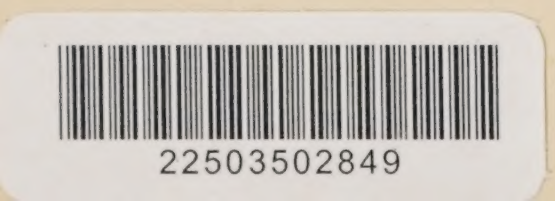
BACTERIAL DISEASE

BIOLOGICAL PRODUCTS IN PROPHYLAXIS AND TREATMENT

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES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INTRODUCTION

BIOLOGICAL products used in bacterial disease may be divided into three groups, viz.

(1) sera of various types for prophylaxis and treatment by passive immunisation,

(2) prophylactics, such as toxins, toxoids and bacterial vaccines for active immunisation,

(3) diagnostic products, such as diluted toxins and tuberculins, used to detect the presence or absence of immunity. In the third group are included certain sera and suspensions used in the laboratory for Wassermann, precipitin or agglutinin reactions.

IMMUNITY

This is of two types and may be *active* or *passive*.

(1) *Active* immunity is immunity acquired by the body itself *(a)* during natural recovery from disease, *(b)* from a latent infection or *(c)* artificially as a result of injections of toxoids, vaccines or other prophylactics. It is due to the formation of *specific* resisting powers against the causal organism or its toxin. Toxoids, toxins or vaccines responsible for the production of specific antibody formation may be termed antigens.

Active immunity, produced by the injection of a prophylactic, takes some time to develop. A single injection of an antigen such as diphtheria toxoid may give rise to only minimal or basal immunity *(" primary stimulus response ")* but in some way it educates the body so that a second inoculation, given after a suitable interval, will produce large amounts of antitoxin rapidly *(" secondary stimulus response ")*.

Active immunity is much more lasting than passive, and even when the antibody is no longer detectable in the subject's serum, there remains a potential immunity throughout life, *i.e.*, the body cells possess latent capacity to respond rapidly to specific stimuli so that adequate antitoxin is produced when required.

(2) *Passive* immunity is established by injecting an *immune serum* or *antiserum* prepared from some other animal and is relatively rapid in onset, the rapidity depending upon the route of injection.

Protection is obtained almost immediately following intravenous injection, more gradually after intramuscular injection, and still more slowly after subcutaneous injection. It has been shown experimentally that twenty-four hours after the subcutaneous injection of a dose of diphtheria antitoxin, the antitoxic titre of the blood is only about half that obtained after an intravenous injection of the same dose.

Passive immunity, however, is of short duration (at the most, a few weeks) owing to the elimination of the foreign protein by the body. When homologous serum is used, as in the prevention of measles, the rate of elimination is much slower.

METHODS OF ADMINISTRATION

The most careful antiseptic precautions should be observed. Many reactions which are ascribed to the serum or prophylactic are really due to bacterial infection, and many published and private reports from British and foreign sources demonstrate the grave risk incurred if practitioners resort to slip-shod methods of sterilisation.

Syringes and needles should be sterilised by heat, either in the autoclave or by boiling for ten to fifteen minutes in one per cent phenol. As a further precautionary measure, special syringes should be reserved solely for biological products and fluids which are bacteriologically " clean." The hands of the operator should be cleansed by scrubbing with soap and hot water.

A simple and efficient method of preparing the site for injection is to rub the part with swabs soaked in methylated spirit, and then paint with iodine. The solvent action of the alcohol on fatty exudate on the skin and in the hair follicles and sweat glands aids the removal of most of the microbes present ; the iodine, even on momentary contact, will probably injure most staphylococci and streptococci.

Before opening an ampoule, any liquid lodging in the neck should be shaken down by a movement similar to that used in setting a clinical thermometer. To open the ampoule, the tube should be broken off at the file-mark near the lower margin of the label. This is best done by holding the ampoule with the file-mark uppermost and with the neck slightly higher than the body. The tips of the thumbs, protected by gauze, are then placed together on the neck just opposite the file-mark and the neck broken off with a sharp snap.

Opinions vary widely as to the best method of sterilisation of the cap of an indiarubber-capped bottle. One method is to wrap a sterile swab, lightly wrung out with 15 per cent lysol, around the cap while the syringes and needles are being boiled, *i.e.*, for 10 to 15 minutes, at the end of which the swab is removed and the cap dried with another sterile swab. Other antiseptics, *e.g.*, 1 in 20 phenol, Dettol, methylated spirit followed by iodine, can be used in a similar manner, the rubber cap of the bottle being dried before the needle is inserted.

It is usual to fill the sterile syringe with a volume of air approximately equal to the quantity of serum to be withdrawn from an indiarubber-capped bottle. The needle is then pushed vertically through the cap, the bottle inverted, the air injected, and the requisite quantity of the serum withdrawn.

Before the injection is made, care should be taken to expel all air from the syringe.

It is important to use only *sharp* stainless steel needles. There is still an unfortunate tendency to use needles which have been blunted by too frequent or careless use. Many needles are ruined by being left too long in disinfectant solutions.

It should be carefully noted that once a sealed ampoule or bottle is opened it is highly undesirable, owing to risk of contamination, to reserve any portion of the contents for future use. The whole of the serum should at once be used on one or more patients.

(1) **Intradermal Injections** for sensitivity or diagnostic tests are usually made about the middle of the front of the forearm. The operator stretches the skin by holding the forearm tightly in his left hand, and slowly inserts the needle, with the bevel upward, for about 2 mm. into the superficial layers of the dermis almost parallel with the surface. A special needle, short and stout with a short bevel, is used and can usually be seen faintly through the epidermis during insertion. A raised blanched bleb showing the pits of the hair follicles is a sign that the injection has not been made too deeply.

(2) **Subcutaneous Injections** should be made in a location where the skin is loose, the tissues yielding and veins scarce. The site most frequently employed is the outer aspect of the arm or the thigh. Such injections may, however, be given between the scapulæ or under the skin of the abdomen. A fold of skin is pinched up with a firm grasp and the needle is pushed sharply, with a slight stabbing motion, through the entire thickness of the skin, into the middle of the fold. The needle is kept parallel with the surface. The injection should be continuous but not rapid, in order that it may be as painless as possible. In the case of a large injection, the needle should be withdrawn slowly as the injection is made.

(3) **Intramuscular Injections** are best made into the deltoid or triceps for small amounts, and into the gluteal region or lateral aspect of the thigh if the amounts are large. The part must be steadied and the skin stretched during insertion of the needle. For gluteal injection the patient may be prone, lying on the side opposite to that of the injection or standing. The prone position is the best, and relaxation of the buttock muscles should be obtained by turning the toes inwards. The point of injection, in the upper and lateral quadrant of the gluteal region, is chosen to avoid striking bone or depositing irritant material on the periosteum or near the

roots of the sacral plexus or sciatic nerve, where it may give rise to pain, induration or obstinate sciatica.

The needle point should have a long bevel and must not be turned by bringing it into contact with hard substances. The needle must be inspected closely to detect any corrosion or weakening so as to avoid any possibility of a fracture at the junction of the mount and the shaft during injection.

(4) **Intravenous Injection** is usually made into a vein at the bend of the elbow. The patient should be asked to hang the arm downwards, clenching and unclenching the hand meanwhile. This helps to make the vessels more prominent. The veins may then be further distended by tying a tourniquet, a piece of rubber-tubing, a sphygmomanometer cuff at about 80 mm. of mercury pressure or a bandage round the upper arm. These appliances should be just sufficiently tight to engorge the veins. After disinfection of the skin in the usual manner the needle, which should have a relatively short bevel, is inserted in a direction parallel with the axis of the vein selected and towards the proximal end of the limb. One must always be sure that the needle is actually in the vein by withdrawing the piston of the syringe slightly when blood appears in the barrel of the syringe. The tourniquet should be removed as soon as the vein has been entered and before the injection is given. Should the needle not have entered the vein at the first attempt, it may be wiser to transfer to the other elbow. The development of a hæmatoma should be avoided by prompt pressure with a pad on withdrawal of the needle.

In young subjects one of the veins on the inner side of the ankle or the external jugular may be more accessible than the median basilic or median cephalic.

Serum should be given very slowly and cautiously. If large volumes of diluted serum are to be given, the solution should be warmed but not above 40° C. as otherwise the protein will coagulate. The " drip " process which is sometimes used has the advantage of minimising shock.

(5) **Intraperitoneal Injection** may be used in severe cases of diphtheria, scarlet fever, etc., in infants and children with inconspicuous veins. Absorption is much more rapid than after intramuscular injection. The serum is usually given in the middle line just below the umbilicus.

(6) **Intrathecal Injection** is still used occasionally in tetanus, cerebro-spinal fever, etc. The patient may be placed in the lateral

position on a firm surface with the knees drawn up and the body arched so as to open the intervertebral spaces. The puncture is usually made in the space between the third and fourth lumbar vertebræ (sometimes in the space between the fourth and fifth). The line connecting the highest points of the iliac crests cuts the spinous process of the fourth lumbar vertebra and the needle is introduced between this and the spinous process above (or below).

In some cases it may be desirable to give a general anæsthetic in order to prevent movement with the needle *in situ* ; in others a few minims of a local anæsthetic solution may be injected under the skin over the area of the puncture. The skin is first carefully sterilised and swabbed with 70 per cent spirit and iodine or other antiseptic. A needle 3 to $3\frac{1}{2}$ inches in length of about S.W.G. 18 and fitted with a removable stilette is required. It is thrust through the skin and muscles into the spinous ligaments in a direction straight forward or slightly upwards in the median line ; then more slowly until a cessation of resistance indicates that the canal has been entered. This usually occurs at a depth of about $2\frac{1}{2}$ to 3 inches in the adult or from $\frac{1}{2}$ to 1 inch in the child. The stilette is now withdrawn and if no spinal fluid escapes the needle should be withdrawn a little or cautiously advanced and the stilette reinserted once or twice to dislodge occluding material. The patient may be instructed to take a deep breath. The required amount of spinal fluid is allowed to escape slowly and then the fluid to be administered, previously warmed to body temperature, is allowed to run in slowly from a filled syringe or preferably by gravity pressure at a rate of about 1 c.c. per minute. If symptoms of collapse occur during the injection, some of the fluid should be allowed to escape at once and the administration stopped or continued with caution. After completion, the needle is rapidly withdrawn and the wound covered with sterile gauze. Strict asepsis of hands and instruments must be maintained throughout the manipulation. In cases where the injected fluid is required to diffuse towards the head *(e.g.*, anti-meningococcus serum), the foot of the bed should be raised for one or two hours.

Intracisternal injection between the base of the skull and the first cervical vertebra is recommended by some clinicians in preference to lumbar injection in cases of cerebro-spinal meningitis. Cisternal puncture should only be carried out by those who have had special training and experience of the method as it is associated with considerable risk.

SERUM DISEASE: ANAPHYLAXIS

Serum sickness is less common than formerly *(see* Refined Sera, foot of page 13), and " accidents " (sudden and severe reactions in persons who are hypersensitive to serum) are very rare. Some knowledge concerning these different manifestations is essential to the physician.

Three groups of symptoms may follow the giving of serum :

(1) the well-known delayed serum reaction with rashes, temperature and joint pains appearing seven to fourteen days later, but sometimes a little earlier,

(2) the immediate intense reaction (anaphylaxis), characterised by shock, collapse and dyspnœa, which comes on possibly within five minutes of injection or gradually in the first few hours,

(3) the severe chill or rigor with a rapid rise of temperature (thermal reaction) following intravenous injection.

It is probable that the first and second groups depend on the same immunological basis, but to the clinician they present themselves as sharply distinct phenomena.

(1) *Delayed Serum Reaction*

The serum reaction appearing about a week after injection is the form most commonly met with. It is due to the development of precipitins before the original dose of serum has disappeared from the body, the injected serum protein acting as an antigen. When unconcentrated serum is injected in ordinary doses, 50 per cent or more of the patients may show some serum reaction ; rash is the prominent feature, in some patients passing almost unnoticed, in others causing intense irritation and severe discomfort lasting several days and demanding active treatment to ensure rest and sleep ; in others severe pains may also be present in many of the joints, with fever, headache, vomiting, slight lymphadenitis and feeling of considerable illness lasting several days. The rash is usually urticarial in type and may be associated with a certain amount of œdema, as of the face. Soothing lotions, antipyretics and adrenaline or ephedrine are the most useful remedies for treatment.

When concentrated sera were introduced, the incidence of serum reactions decreased to probably between 10 and 25 per cent, the higher incidence occurring when large doses were given ; this

diminution in incidence was due to reduction in the amount of protein administered. With the further improvement in methods of preparation represented by the " refined " sera, the incidence of serum rashes fell to very much lower proportions ; over a large series of cases the number of serum reactions averaged less than 5 per cent. This striking reduction is due not only to the further large diminution of the amount of protein for each unit of antitoxin but to a modification of the molecular structure of the refined antitoxin, rendering it a much poorer antigen in the production of precipitin.

Very rarely, when a second injection of serum is made during the course of serum disease, swelling and marked induration with sloughing of both subcutaneous and muscular tissues have been observed. This is analogous to the Arthus phenomenon in animals and is apparently due to a local skin hypersensitivity. The onset of this rare phenomenon is usually gradual. To prevent its development it is recommended by some authorities that a second intramuscular or subcutaneous injection of serum should not be made near the site of a previous injection.

Attention has been directed during recent years to rare neurological sequelæ of serum treatment. Neuritis of various forms, local paralysis and sometimes cerebral symptoms have been described. The prognosis is good in most cases.

(2) *Immediate Serum Reaction or Anaphylaxis*

There are people who are intensely sensitive to horse serum ; many asthmatic patients are in this group. Although acutely susceptible patients may die within five minutes of the intramuscular or subcutaneous injection of perhaps 1 c.c. of serum, the danger is greatest when the serum is given intravenously. Fortunately, these sensitive subjects are very rare ; estimates of 1:50,000 to 1:100,000 have been made. Fear of anaphylaxis is no sound justification for refusing or delaying the administration of antitoxin to any patient needing it.

Usually dyspnœic symptoms are prominent but in a few instances pallor and collapse have been recorded. When this acute immediate reaction begins to appear, adrenaline (0·5 c.c. of 1:1000 solution) should be given immediately, either intramuscularly or subcutaneously, and repeated if recovery does not occur rapidly. In desperate cases physicians have injected it intracardially. Ephedrine has recently come into favour, and atropine has also been used. If the serum has been injected into an extremity, a tourniquet may be applied for a short time above the site of injection.

(3) *Thermal Serum Reaction after Intravenous Injection*

This is to be distinguished from the immediate shock-like reaction described on *page* 10. Within half-an-hour of injection a rigor may come on which usually gives rise to little anxiety and responds readily to the usual measures, *e.g.*, hot bottles, raising the foot of the bed and the injection of adrenaline.

The Prevention of Serum Reactions

All adults and older children should be questioned about previous injections of serum and the history of asthma or hay fever in the patient or family.

The intradermic injection of one drop of diluted serum has been employed as a test of sensitivity but the results have been unreliable. The conjunctival reaction to a drop of a 1:10 dilution of serum has also been used. Diffuse congestion has been taken as an indication of sensitivity, but here again the test has proved of little practical value.

In view of the unreliability of these tests, the safest course is to give a small dose, for example 0·1 or 0·5 c.c. of a 1:10 dilution of serum in sterile saline, by the same route as that which has been chosen for the main dose. If no disturbing symptoms appear within half-an-hour the main injection may then be given very slowly.

Reactions may follow the administration of serum by any route, but are commoner after intravenous injection. It is probably true that the great majority of intravenous injections of serum are given only to patients gravely ill. The physician will therefore usually feel that the risk of complications is less than the risk of delay in giving the remedial serum and will inject the main dose of serum without preliminary test, commencing the injection with extreme slowness and stopping immediately if any untoward symptoms develop.

As a result of animal experiment, it was formerly thought that sensitised patients could be " desensitised." It is doubtful to what extent this can be done.

The precautions to be observed in the intravenous administration of serum are *(a)* a warmed serum, *(b)* a very slow injection, *(c)* careful observation of the patient, *(d)* hypodermic preparations of adrenaline and possibly atropine instantly available, and *(e)* a tourniquet ready for application above the site of injection.

SERA

Sera may be arranged conveniently in three groups, viz.

(1) antitoxic sera, such as diphtheria, hæmolytic streptococcus, staphylococcus, dysentery (Shiga), tetanus and gas gangrene antitoxins ;

(2) anti-bacterial sera, such as anti-pneumococcus, anti-meningococcus and anti-leptospira sera ;

(3) anti-viral sera, represented by convalescent (human) measles, convalescent yellow fever, anti-poliomyelitis, anti-influenza and anti-dog distemper sera.

ANTITOXIC SERA

Some organisms produce poisonous substances in the media in which they are grown. These poisons or toxins can be demonstrated in the media after the organisms have been removed by filtration and are referred to as "soluble toxins" or "*exotoxins.*" Their activity can be determined by injection into animals.

Toxins in this class can be rendered atoxic or harmless by adding formalin and incubating the mixture, but they retain their antigenic or immunising properties ; filtrates detoxicated in this way are known as *toxoids* or *formol-toxoids.* If an animal is injected with non-lethal doses of toxin or with the corresponding toxoid, it develops in its serum specific antibodies or *antitoxins.*

In practice in serum laboratories horses are injected with repeated and gradually increasing doses of the appropriate antigen usually prepared from special toxigenic strains. Small specimens of blood are withdrawn from time to time and the titre of antibody estimated in the serum by titration against the appropriate toxin. When a satisfactory titre has been reached larger amounts of blood are withdrawn.

The serum which contains the antitoxin is subjected to stringent laboratory tests and is usually concentrated. As issued to the medical profession it must be of satisfactory potency, non-toxic and

sterile. It usually contains a small amount of antiseptic, *e.g.*, tricresol. When the methods available permit, the serum is standardised to contain a certain number of units per c.c., the unit being a specified quantity of a standard antitoxic serum.

Therapeutic Substances Act.—The potency, sterility and absence of toxicity of sera, vaccines, prophylactics and certain other therapeutic agents such as insulin and pituitary extract are controlled in Great Britain by regulations issued by the Ministry of Health under the Therapeutic Substances Act. Manufacturers of such products work under a licence issued by the Ministry and such licences may be revoked if the requirements of the Act are not complied with. Periodical tests of products bought in the open market are made for the Ministry. The regulations include specific requirements as to the nature of the tests made. For diphtheria, tetanus, gas gangrene and staphylococcal antitoxins, anti-dysentery serum (Shiga) and anti-pneumococcal sera, samples of carefully dried standard serum in sealed ampoules are kept for the Ministry at the National Institute for Medical Research at Hampstead.

These standard sera are available to manufacturers in order that they may prepare their own laboratory standards of potency. The British standard sera are themselves carefully compared with International standards for the League of Nations Health Commission and distributed, in normal times, from the State Serum Institute at Copenhagen. Legislation similar to the British legislation has been adopted in many other countries and the International standards are available there also, so that sera prepared in all parts of the world shall be of the same standard of potency.

Concentration and Purification of Antitoxic Sera.—Methods have been developed which separate the antitoxin-bearing globulin from those proteins which, while adding to the bulk of the preparation, are devoid of antitoxic value. Non-antitoxic or normal proteins are probably at least equally responsible for those incidental toxic symptoms which sera may produce in susceptible patients. The use of concentrated sera therefore reduces the quantity of serum to be used and also the risk of serum sickness. Until recently, fractional precipitation with ammonium sulphate or sodium sulphate was the method most used for the concentration of antitoxic sera. Selective treatment of serum with proteolytic enzymes results in removal of unwanted protein and modification of the residual globulin. This marks a further important advance in the reduction

of the incidence of serum sickness ; over a large series of cases the number of serum reactions averaged less than five per cent. The volume to be injected is still further reduced. For the same number of units enzyme-treated serum contains less than half the amount of protein present in the concentrated sera hitherto used. The antitoxin-bearing molecule is smaller, a property which appears to be connected with the low liability of enzyme-treated antitoxin to cause serum sickness and also with the greater efficiency of the serum.

Serum treated in this way with proteolytic enzymes is referred to as "refined" serum in the following pages.

ANTI-BACTERIAL SERA

Sera are available against bacteria which do not produce poisonous filtrates analogous to the true "soluble toxins" of diphtheria or tetanus. Organisms such as the pneumococcus, meningococcus and *Leptospira icterohæmorrhagiæ* give rise to toxins which are essentially intracellular and are liberated as a result of autolysis in old cultures or in the animal body. When the organisms themselves, either dead or alive, or certain of their products, are injected into horses, antibodies may be produced which lead to the disintegration of the bacteria. Anti-bacterial sera appear to act by neutralising the virulence of the organisms and rendering them an easy prey to phagocytosis. The sera are treated and tested by appropriate methods analogous to those used for ensuring the potency and safety of antitoxic sera.

Certain anti-bacterial sera may be concentrated by special methods, which differ from those used for antitoxic sera since the essential antibodies are contained in different fractions. Thus, in the case of concentrated anti-pneumococcus sera it is the water-insoluble proteins which are retained by the Felton process.

ANTI-VIRAL SERA

Serum from human convalescent patients is of considerable value in the prophylaxis of *measles*. Amounts of 5 to 10 c.c. given before the fifth day after exposure will often prevent the disease, and given after the fifth day will so attenuate or modify it that the risk of complications is greatly reduced. Complete prevention of an attack is followed by full susceptibility in a few weeks or months when the homologous serum has been excreted, but a modified attack ensures an active and therefore more or less permanent immunity.

When convalescent serum is unobtainable, the serum of healthy young adults who have had measles in childhood may be substituted. Larger doses are necessary, *e.g.*, 12 to 30 c.c. In an emergency, 25 to 50 c.c. of whole adult blood from parents or other compatible donors has proved effective.

The serum of patients convalescent from *yellow fever* has strong protective properties against yellow fever infection. Advantage is taken of this in the mouse protection test in yellow fever whereby the survival or otherwise of mice injected simultaneously with virus and serum being tested will indicate whether a previous illness was yellow fever or not. The immune serum is not used for treatment, although a vaccine in the form of living attenuated virus grown in tissue culture or in chick embryo is extensively employed as a prophylactic.*

The evidence for the use of human anti-viral serum in *poliomyelitis* is less convincing than in measles. It is also difficult to draw conclusions as to the efficacy of anti-viral sera prepared in the horse.

Serum obtained by immunising horses with the virus of *influenza* neutralises or inactivates the virus in mice. Its use is still in the experimental stage, and difficulties have arisen from the existence of several types of virus differing in antigenic characters.

An immune serum obtained from dogs injected with *distemper* virus is of considerable value in veterinary practice.

*NOTE.—Yellow fever vaccine is made in The Wellcome Bureau of Scientific Research at The Wellcome Research Institution, Euston Road, London, as part of a research in virus disease which has been in progress for some years. Owing to the delicate nature of the vaccine, which has to be kept under special conditions, it is unsuitable for general distribution. However, persons proceeding to parts of the tropics where danger of yellow fever infection exists may, if they so desire, be vaccinated free of charge at The Wellcome Research Institution.

Keeping Qualities of Sera

In the case of certain antitoxic sera, *e.g.*, the refined preparations for use in diphtheria, dysentery (Shiga), tetanus and gas gangrene, the experimental determination of the activity can be made with such precision that the rate of deterioration can be determined with accuracy. In these cases, therefore, it can be stated with certainty that the sera, if kept under the indicated conditions of cool storage, will be of at least the stated value on the date indicated and may be expected to deteriorate at a very slow rate thereafter, so that they may be used with confidence for a considerable period after the date in question. There is no reason for supposing that different types of antitoxic sera vary in their stability.

In the case of anti-bacterial and anti-viral sera the available evidence of the rate of deterioration has not the same precision, but there is reason to believe that these sera are somewhat less stable than antitoxic sera.

The regulations of certain countries require a time limit upon the package but it is not meant to imply that the serum is useless after the date quoted.

The rate of deterioration of sera increases with the temperature of storage, and sera, especially refined sera, kept at from 2° C. to 4° C. may be expected to be of full labelled value almost indefinitely. Kept at 15° C., the rate of deterioration is less than 10 per cent per annum. *It is obligatory for manufacturers to add excess units to all bottles or ampoules, the labelled unitage being always less than the actual unitage in the container by an amount which allows for deterioration at ordinary room temperature before the expiry date.* In a hot cupboard or in the tropics deterioration may easily exceed 25 per cent per annum.

N.B. The optimum temperature for storage is just above freezing point, *i.e.*, 2° to 4° C.

Although actual freezing of serological products should be avoided, the loss of potency of *undiluted* serum if this occurs is extremely small and much less than that due to exposure for any length of time to high temperatures.

Certain products are *diluted* so that the dose is contained in a volume suitable for injection; it is most important that these preparations should not be frozen solid. When frozen there is a high local concentration both of protein and of antiseptic. The increase in concentration of the antiseptic may cause considerable deterioration of the serum.

ANTITOXIC SERA

DIPHTHERIA ANTITOXIN

Diphtheria antitoxin is prepared by immunising horses against specific diphtheria toxin and is titrated in the laboratory by injecting mixtures of toxin and antitoxin subcutaneously and intradermally into guinea-pigs. There is also a subsidiary *in vitro* flocculation method involving the mixture of a constant amount of toxin or toxoid with falling amounts of antitoxin. Titrations are always performed with reference to the standard antitoxin *(see page* 13).

Use in Prophylaxis.—Doses of 1000 to 2000 units of refined antitoxin may be administered to the rest of the household in which a case of diphtheria occurs and to any other persons who have been in recent contact with the patient, particularly those who have given a Schick-positive reaction. It must be remembered, however, that the passive immunity produced by the injection is of a temporary nature only, lasting on the average, from two to three weeks. Active immunisation with diphtheria prophylactic A.P.T. or T.A.F. may therefore be commenced during the second week or possibly injections of the serum and of the first dose of prophylactic may be given concurrently soon after exposure to infection. Further injections of the prophylactic may be given at intervals of three to four weeks. In certain circumstances another procedure has been followed; the persons are immunised actively with diphtheria prophylactic and carefully watched for signs of diphtheria, serum being injected only if sore throat develops.

Use in Treatment.—Serum should be given at the earliest possible moment and, preferably, in one adequately large dose.

Neither the initial administration of antitoxin nor the repetition of the dose should ever be delayed for the result of a bacteriological examination.

Mild cases of diphtheria may be given 2000 to 10,000 units, 2000 units being reserved for cases under constant supervision in hospital; otherwise 8000 to 10,000 units should be the minimum dose. The dose for a case of moderate severity seen early in the disease should never be less than 15,000 units and in more severe cases, or in moderate cases seen after the third day, 30,000 to 100,000 units should be injected at once. These doses are based on the L.C.C. Departmental Report on Dosage of Antitoxin in Diphtheria and should be given irrespective of age, because the case mortality from diphtheria in young children is high. In urgent cases the

serum should be given intravenously if possible, although the practice of some authorities is to give part of the serum intravenously and part intramuscularly. When intravenous injection is difficult but desirable the intraperitoneal route may be selected. Alternatively in infants the dose may be given into the superior longitudinal sinus before closure of the fontanelle. When the illness is less acute the intramuscular route may be chosen—or rarely the subcutaneous in persons sensitive to serum in whom slow absorption may be desirable.

STREPTOCOCCUS ANTITOXIN (SCARLATINA)

This antitoxin is prepared by injecting horses with toxins of *Streptococcus hæmolyticus scarlatinæ.*

The method of titration used at The Wellcome Physiological Research Laboratories is based on intradermal tests in rabbits carried out in comparison with a standard serum issued by the official U.S.A. authority at Washington. An *in vitro* flocculation method similar to the one used for the titration of diphtheria antitoxin has been introduced recently.

No official British standard serum or unit has been prescribed but the minimum level of potency adopted for issue corresponds with American antitoxin containing 1000 U.S.A. Units per c.c. The indicated potency of 'Wellcome' Streptococcus Antitoxin is based on a conservative interpretation of the assay made. The titration of scarlatina serum has nothing like the precision of the titration of diphtheria serum and exaggerated claims of potency are made for some foreign sera.

Use in Prophylaxis.—Passive immunisation with refined antitoxin has been found of value when scarlet fever has occurred in general wards in children's hospitals, schools and institutions, and amongst diphtheria convalescent patients. All the contacts are examined by the Dick Test *(see page* 36) and those giving a positive reaction are immediately protected with antitoxin. The usual dose is 3000 U.S.A. Units, which will turn most "Dick-positive reactors" negative within 24 hours and keep the reaction negative for some days. Some clinicians prefer to give 1500 U.S.A. Units. Clinical experience has shown that by this method the majority of contacts can be efficiently protected against streptococcal toxin. It should be remembered, however, that they are still susceptible to tonsillitis, etc., and may have to be isolated.

Some authorities recommend that if the chances of contracting scarlet fever are not high, active immunisation should be chosen in preference to the administration of serum *(see page* 38).

Use in Treatment.—In scarlet fever the antitoxin has an obvious curative effect, if given early enough and in proper dosage. In the average patient the temperature falls rapidly and the clinical condition is much improved within a few hours of the administration of the serum. Many clinicians believe that the incidence of serious complications is also reduced, but this view is not universally accepted. The probability is that the antitoxin, by enabling the patient to overcome the toxæmia, allows the natural defences of the body to combat the pyogenic or septic complications. As scarlet fever antitoxin is effective essentially against the erythrogenic toxin, it is of little or no use in fevers without rash, *e.g.*, septicæmia, puerperal fever, tonsillitis, etc.

Many physicians do not give serum to mild cases of scarlet fever, preferring to avoid the risk of serum sickness. If it is thought advisable to treat mild cases, 3000 U.S.A. Units would seem a suitable dose ; experience seems to suggest that not less than 10,000 U.S.A. Units should be given to severe cases, repeated daily if necessary, and from 10,000 to 30,000 U.S.A. Units to very severe cases. The dose recommended by the Scarlet Fever Committee is at least 8000 U.S.A. Units. The serum is given intramuscularly in ordinary cases and intravenously in very urgent cases. In young children the intraperitoneal route on account of the relative ease of administration has been advocated.

ANTI-STREPTOCOCCUS SERUM, ERYSIPELAS
ANTI-STREPTOCOCCUS SERUM, POLYVALENT
ANTI-STREPTOCOCCUS SERUM, PUERPERAL FEVER

These three sera are prepared by injecting horses with hæmolytic streptococci and their products, the organisms being obtained from subjects suffering from the respective diseases. They have been issued only in response to the wishes of those who feel that the sera have produced favourable results. The amount of antitoxin present is much less than in scarlet fever antitoxin, and most physicians, if they decide to use a serum in the treatment of any infection caused by hæmolytic streptococci, will inject the refined scarlet fever antitoxin.

Unless there is a rash it is very questionable whether any of the sera now available should be given. Drugs of the sulphanilamide group are much more likely to be useful. However, certain cases of traumatic erysipelas and puerperal fever with rash respond well to treatment with antitoxin. When it is decided to use serum in any

of these infections it would appear wise to use large doses, *e.g.*, 50 c.c., preferably by the intravenous route.*

STAPHYLOCOCCUS ANTITOXIN

This serum is obtained by immunising horses with toxin and toxoid from certain strains of staphylococcus. It neutralises the lethal, skin-necrosing and hæmolytic properties of the toxin and can thus be compared with the standard preparation.

The exact role of staphylococcus antitoxin in immunity to human disease is not yet understood. Although persons who have had staphylococcal lesions may have a high titre in their blood, there are many exceptions to this rule, and the relation of antitoxic titre to disease is often very erratic. In clinical practice there is some evidence that the antitoxin has a favourable influence on the septic or pyogenic effect of the staphylococcus, although the results have not been very striking.

In septicæmia or acute infection (osteomyelitis, boils, carbuncles, etc.) with toxæmia, the refined antitoxin may be given intravenously at the earliest possible moment in a dose of at least 10,000 to 20,000 units, repeated after 12 or 24 hours. In less urgent cases it may be given intramuscularly.

ANTI-DYSENTERY SERUM (SHIGA)

This serum is a true neutralising antitoxin prepared by immunising horses with dysentery (Shiga) toxin and toxoid and is best titrated in the laboratory by injecting mice intravenously with mixtures of serum and a suitable lethal toxin. The protective effect of the serum is thus determined and compared with that of the standard antitoxin.

The anti-dysentery serum now available has a much higher antitoxin content than that used during the war of 1914–1918. In many cases treated with serum there has been a dramatic improvement in symptoms, including the development of a sense of well-being, reduced temperature and toxæmia, marked and immediate decrease in the number of stools and amelioration of tenesmus and colicky abdominal pain. When cases have been treated at a reasonably early stage of the disease the mortality has been low.

* Sulphonamide-P (Sulphanilamide) is of considerable value in the treatment of hæmolytic streptococcal infections, with the exception of early scarlet fever, the toxæmia of which appears to be uninfluenced. The drug is available as ' Tabloid ' Sulphonamide-P for oral administration and as ' Hypoloid ' Sulphanilamide L.S.F. for parenteral administration. *(See special literature.)*

The serum may be given in doses of 10,000 to 100,000 units intravenously (or less effectively intramuscularly), repeated daily if necessary.

TETANUS ANTITOXIN

Tetanus antitoxin is prepared by immunising horses with toxin and toxoid. The usual method of titration in the laboratory involves the injection of mixtures of toxin and antitoxin into mice or guinea-pigs. The specific capacity of the serum to neutralise the toxin is compared with that of a standard preparation and a unit value thus allotted.

This serum has proved less effective in the treatment of tetanus than diphtheria antitoxin in diphtheria. On the other hand, as a prophylactic, it has proved invaluable in the case of wounds contaminated with soil, dirt, etc., particularly in areas where tetanus is encountered. Its value in this respect was thoroughly demonstrated in the war of 1914–1918. When given early after wounds were inflicted the serum protected against tetanus, the incidence of which was considerably reduced. Where the serum was not given sufficiently early to prevent tetanus, the attack occurred only after a prolonged incubation period and was mild in type with a low death rate.

Use in Prophylaxis

(a) After receipt of an injury—

The subcutaneous injection of 1000 to 3000 International Units* of antitoxin within a few hours of the receipt of an infected wound materially reduces the possibility of the subsequent onset of tetanus. The larger dose is strongly recommended by the Medical Research Council (*British Medical Journal*, April 8th, 1939, *p.* 738). The protection conferred is at its maximum two to three days after the injection, and slowly diminishes, becoming very slight after three weeks. Where very severe injuries exist and a large amount of dead tissue is present, which favours the rapid growth of tetanus bacilli and the consequent production of large amounts of toxin, the injection of a second prophylactic dose of serum, given seven to ten days after the first dose, may be considered advisable to prolong the period of protection by "passive immunisation." Many clinicians now inject a mixture of tetanus antitoxin and gas-gangrene antitoxin as a prophylactic after wounds which may have been infected by both organisms. (*See page* 25.)

* The International Unit, which has been accepted in most countries, is half the U.S.A. Unit. Thus 2000 units in the new nomenclature is equal to 1000 units hitherto used in English-speaking countries, *i.e.*, the official U.S.A. Unit. Probably for some years to come both units will be expressed together in English literature.

Wherever tetanus is common a dose of either tetanus antitoxin or tetanus and gas-gangrene polyvalent antitoxin should be given subcutaneously as soon as possible after the occurrence of wounds, compound fractures, etc., which have become soiled with dirt of any kind, and also before emergency operations performed under conditions which make asepsis impossible. In places where tetanus neonatorum causes a heavy mortality the use of antitoxin has been suggested, and where there is reason to suspect that the umbilical wound has not been treated with surgical cleanliness, a dose of antitoxin would seem to be strongly indicated.

(b) Before operation—

i The occurrence of tetanus among a number of soldiers operated on long after being wounded in the war of 1914–1918, induced military surgeons to make a routine of injecting a dose of serum before operations involving tissue previously injured.

ii Veterinary surgeons who make a practice of injecting 2000 International Units of antitoxic serum before operations, such as castration, docking, etc., in districts where tetanus is common, find that such an injection ensures almost complete protection against the occurrence of tetanus in the animals operated on.

N.B.—The immunity conferred passively by antitoxic serum may be reinforced actively by tetanus toxoid *(see page* 41), giving the first injection two weeks after the dose of antitoxic serum and the second and possibly third injections at intervals of four to six weeks. Alternatively, injections of the antitoxic serum and of the first dose of toxoid may be given concurrently soon after exposure to infection. Further injections of toxoid may be given at intervals of four to six weeks.

Use in Treatment.—Though, unfortunately, it is often too late for antitoxin to have effect when the patient first comes under medical care, it should be given in large doses at the earliest possible moment after the diagnosis is evident or reasonably probable. Careful watch should be kept for any accentuation of tendon reflexes following a soiled wound, and pain so severe as to be out of proportion to the injury should be deemed suspicious.

Most authorities now inject 100,000 to 200,000 units *intravenously*, the injection being repeated if necessary until symptoms abate. The intrathecal route has been largely abandoned; some authorities have pointed out that if serum has to reach the motor cells by the blood stream there should be no reason for intrathecal

administration. It has also been stated that this route may be definitely harmful.

There is no reason why other treatment considered advisable, such as the liberal use of bromides, chloral, morphine, barbiturates, etc., should not go on simultaneously with the antitoxin treatment.

GAS-GANGRENE ANTITOXIN

Gas-gangrene in man is caused by one or more members of the genus *Clostridium* or sporing anaerobes, of which the most important are *Cl. perfringens (Clostridium welchii*, type A), *Cl. septique*, *(Cl. œdematis maligni*, *Vibrion septique)* and *Cl. œdematiens (Cl. novyi)* ; these three organisms were identified in actual cases of gas-gangrene in the war of 1914–1918. Of less importance are *Cl. histolyticum* and *Cl. sordelli.*

The three following antisera are commonly used in this country:—

(1) gas-gangrene antitoxin (perfringens) ;

(2) gas-gangrene antitoxin (polyvalent) which contains a mixture of antisera to the three main infecting types, viz., *Cl. perfringens*, *Cl. septique* and *Cl. œdematiens* ;

(3) tetanus and gas-gangrene polyvalent antitoxin.

For use overseas, antitoxins to *Cl. histolyticus* and *Cl. sordelli* are sometimes included in gas-gangrene polyvalent antitoxins.

The Ministry of Health Emergency Medical Service recommends the use of the polyvalent antitoxin in cases in which it is desired to combine serum prophylaxis with surgical treatment.

For serum treatment the combined preparation is again recommended ; and, in addition the Service has arranged to supply the separate antitoxins in cases in which the causal organism has been identified. *(British Medical Journal*, 1939, II, Supplement, *page* 169.)

Further data concerning the three sera mostly used are as follows :—

GAS-GANGRENE ANTITOXIN (PERFRINGENS)

This antitoxin is prepared by injecting horses with *Cl. perfringens* toxin, type A, and is titrated in the laboratory by several methods. Mixtures of toxin and antitoxin are injected intracutaneously into guinea-pigs and intravenously into mice, and the neutralising

power of the serum thus ascertained with reference to a standard antitoxin. There is also a hæmolytic test *in vitro*.

Use in Prophylaxis and Treatment.—This antitoxin has been used as a prophylactic against gas-gangrene in grossly infected wounds, such as occur in road accidents, and in the treatment of gas-gangrene occurring with puerperal sepsis after abortion, and also in the treatment of patients with wounds infected with *Cl. perfringens (=Cl. welchii)*. In addition its use has been advocated in abdominal surgery for the prophylaxis and treatment of the toxæmia of acute intestinal obstruction. It must be admitted that though the serum has been in general use for several years there is no general agreement amongst abdominal surgeons on the part played by *Cl. perfringens* in this form of toxæmia or on the value of the serum in treatment. Some are convinced from repeated clinical experience that it has saved their patients ; while others, especially in America, state that there is no experimental or clinical evidence to warrant its use.

The suggested prophylactic dose in soiled wounds is 3000 to 4000 units given intramuscularly. Multiple injections into the muscles in the region of the wound have been recommended by some authorities. When the serum is used therapeutically in gas-gangrene it should be given immediately in large doses, *e.g.*, at least 10,000 to 20,000 units intravenously and possibly also intramuscularly in the region of the infection. Those surgeons who believe in the use of the antitoxin in the treatment of intestinal obstruction give a large dose intravenously and 4000 to 10,000 units intramuscularly daily, until the bowels are acting regularly.

GAS-GANGRENE ANTITOXIN (POLYVALENT)

This serum is prepared by immunising horses against the toxins of *Cl. perfringens (=Cl. welchii)*, *Cl. septique*, and *Cl. œdematiens*. The usual methods of titration involve the injection of mixtures of the separate antitoxins and the appropriate toxins into mice or guinea-pigs.

Use in Prophylaxis.—The provisional proposal of the Ministry of Health Emergency Medical Service is to give subcutaneously or intramuscularly 3000 units of *Cl. perfringens*, 1500 units of *Cl. septique* and 1000 units of *Cl. œdematiens* antitoxin respectively, as a combined injection to cases in which the surgeon desires to combine serum prophylaxis with surgical treatment.

Use in Treatment.—The minimum dose should be 7500 units of *Cl. perfringens*, 3750 units of *Cl. septique* and 2500 units of *Cl. œdematiens* antitoxins. This dose may be repeated.

Since gas-gangrene progresses very rapidly, it is best to administer the antitoxin intravenously, preferably diluted with two volumes of saline and warmed to not above 40° C. If this is not possible, the undiluted material should be given intramuscularly or subcutaneously.

TETANUS AND GAS-GANGRENE POLYVALENT ANTITOXIN

Each phial contains 3000 International Units of antitoxin for *Cl. tetani*, 3000 units for *Cl. perfringens (=Cl. welchii)*, 1500 units for *Cl. septique* and 1000 units for *Cl. œdematiens*. These amounts are in accordance with the provisional recommendations of the Ministry of Health Emergency Medical Service for the serum prophylaxis of tetanus and gas-gangrene.

Use in Prophylaxis.—The entire dose may be injected subcutaneously or intramuscularly as soon as possible after receipt of a wound that may have been infected with pathogenic anaerobes.*

N.B.—The Ministry of Health recommends that an entry of the dosage of antitoxin injected should be made at once on the casualty card or case sheet. This is applicable to all tetanus and gas-gangrene antitoxins.

* Members of the sulphanilamide group, either alone or in conjunction with antitoxic serum, are being used in the prophylaxis and treatment of gas-gangrene *(see special literature sent on request)*.

ANTI-BACTERIAL SERA

ANTI-PNEUMOCOCCUS SERUM

This serum is prepared by immunising horses with killed suspensions of virulent pneumococci (Types I and II respectively) and standardised in the laboratory by injecting varying doses of the serum and a constant dose of living virulent pneumococci intraperitoneally into groups of mice. The protective value of the unknown preparation is compared with that of a standard serum of the appropriate type.

Use in Treatment.—Approximately half of all cases of lobar pneumonia in various parts of the world are caused by Pneumococcus Types I and II. Therefore many physicians wish to give mixed I+II serum immediately the patient is seen. Some workers prefer to give 20,000 to 30,000 units of each antibody as a preliminary dose.

The type of the infecting coccus should be ascertained at once *(see page* 27), and if it belongs to Type I or II, the appropriate concentrated serum is used for treatment.

Specific sera have been used in America and in England, and clinicians are convinced that they (especially Type I Serum) are effective in reducing temperature and producing rapid improvement in the general condition of the patient, thus leading to a significant reduction in the mortality of the disease. They have been employed in all infections—" lobar " pneumonia or " bronchopneumonia " due to the pneumococcus.

A therapeutic dose of 10,000 units may be given intravenously at the earliest possible moment, to be followed by a further 20,000 units one hour later. In toxic cases these initial doses are followed by 20,000 units every three to four hours until at least 100,000 units have been given in Type I cases or as much as 140,000 units in Type II cases. Authorities recommend that treatment may be continued until the temperature falls and the patient's condition improves. The tendency in recent American work is to use high doses ; this is of particular importance more especially where blood culture shows a considerable number of cocci. In these cases some workers use double the ordinary dosage or reduce the interval between injections to two hours.

The sera available are :—

ANTI-PNEUMOCOCCUS SERUM, TYPE I, CONCENTRATED
ANTI-PNEUMOCOCCUS SERUM, TYPE II, CONCENTRATED
ANTI-PNEUMOCOCCUS SERUM, TYPES I+II, CONCENTRATED

These contain the water insoluble fractions of anti-pneumococcus serum found by Felton to represent most of the essential antibody.

Anti-pneumococcus Serum, Type I, Concentrated, and Type II, Concentrated, are each issued in containers of 20,000 units in 12 c.c. or less. Anti-pneumococcus Serum, Types I+II, Concentrated, is a mixed preparation, issued in containers of at least 10,000 units of each type. It is available for physicians who wish to give mixed I+II serum immediately the patient is seen.

Typing of Strain

When it has been decided to use serum for the treatment of pneumonia the infecting strains should be typed as soon as possible. AGGLUTINATING SERA for the diagnosis of pneumonia, Type I and Type II, are available and may be used as follows :—

(1) Neufeld method (adapted to the examination of sputum by Armstrong, and by Logan and Smeall). This method consists essentially in observing in a wet preparation the swelling of the pneumococci and the highly refractile zone which appears round the cocci in sputum when mixed with the specific type serum. The sputum should be as fresh as possible, although the diagnosis of type can usually be made after 48 hours. Thorough mixing of sputum and serum is essential and a cover-slip should be applied at once to prevent drying. A positive reaction occurs within three minutes.

Some workers prefer to add a drop of methylene blue to the mixtures of sputum and anti-serum. The swollen capsules remain unstained.

The Neufeld technique may be applied to pneumococci in blood broth cultures, material from lung punctures, peritoneal exudates aspirated from mice, etc., but here the results are not so satisfactory.

(2) Mouse method. The sputum, obtained preferably after thoroughly rinsing the mouth prior to coughing, is washed in several changes of sterile saline ; a piece about the size of a pea is rubbed up with saline and injected intraperitoneally into a mouse. Four to five hours later a small amount of peritoneal exudate may be aspirated by means of a sterile hypodermic syringe or capillary pipette and distributed in droplets on a slide. Small amounts of *(a)* Type I serum, diluted 1 : 10, *(b)* Type II serum, diluted 1 : 10, and *(c)* saline are added respectively to each of these droplets, and mixed quickly by circular rubbing with a pipette

point. After about two minutes the slide is dried, fixed and stained. If " positive " for Type I or II, specific agglutination will be observed.

If the result is negative or doubtful, the peritoneal exudate can be examined later, *e.g.*, on the death of the mouse which occurs usually after 7 to 24 hours. Details of the macroscopic agglutination method which is then performed are given in manuals of bacteriology.

ANTI-MENINGOCOCCUS SERUM

This serum is obtained from horses injected with strains representing the various types of the *Neisseria meningitidis*, isolated from cases of acute cerebro-spinal meningitis. Laboratory tests are applied to all batches issued although there is no generally accepted method of standardisation of the serum.

Use in Treatment.—The serum is given preferably by the intravenous route, since general systemic infection with septicæmia plays an important part in the illness and often precedes infection of the meninges. Large doses, *e.g.*, 25 to 100 c.c., are injected at the earliest possible moment and repeated if necessary after 24 and 48 hours. Where intravenous administration is impracticable the intrathecal, intramuscular or intraperitoneal routes may be chosen. The amount of serum injected intrathecally should always be less than the volume of cerebro-spinal fluid withdrawn.*

ANTI-LEPTOSPIRA SERUM

This serum, for the prevention and cure of leptospiral jaundice in human beings, is prepared from horses injected with *Leptospira icterohæmorrhagiæ* or its products and will protect animals in controlled laboratory tests against lethal doses of living leptospira culture.

Use in Treatment.—The serum has not been used extensively in the treatment of the human disease in England, but since it represents the only specific treatment, its use is justifiable in the considerable number of cases of this infection revealed by the investigations of the past few years.

* Members of the sulphanilamide group, either alone or in conjunction with antiserum, are being used with striking success in the treatment of pneumococcal and meningococcal infections.

Carefully controlled animal experiments show that the best results are obtained when it is given as soon as possible after infection.

A few human cases are on record in which the giving of serum to a patient, even after deep jaundice was apparent, was followed by a rapid clinical improvement.

Insufficient experience is available to prescribe dosage with any dogmatism. It is suggested that if the infection is severe or if the treatment is late in the disease, 30 to 40 c.c. should be given intravenously. If the serum is being used very early after infection, perhaps the intramuscular route would be chosen, the dose being 20 c.c.

NORMAL HORSE SERUM

This serum has been used locally, internally and subcutaneously, in the treatment of various conditions such as anæmia, hæmophilia, melæna neonatorum, purpura hæmorrhagica, gastric and duodenal ulcer, intestinal hæmorrhage in typhoid fever and tuberculous hæmoptysis. It has also been used as a dressing for ulcers and suppurating wounds.

The doses recommended for injection have been 10 to 20 c.c. for infants and children, and 20 to 50 c.c. for adults.

SEROLOGICAL PRODUCTS FOR DIAGNOSIS AND ACTIVE IMMUNISATION

SCHICK TEST AND ACTIVE IMMUNISATION AGAINST DIPHTHERIA

SCHICK TEST

Under the Therapeutic Substances Regulations (1931), the test toxin must be tested for potency and sterility before issue. The test dose, which is contained in 0·2 c.c., is measured by the following tests :—

(a) by intracutaneous injection into normal guinea-pigs in mixtures with different proportions of diphtheria antitoxin. One test dose mixed with 1/750 or more of a unit of antitoxin must cause no local reaction, but mixed with 1/1250 or less of a unit of antitoxin must cause a definite local reaction of the type known as the " positive Schick reaction."

(b) by intracutaneous injection into normal guinea-pigs, without admixture with antitoxin. One-fiftieth of one test dose must not cause, and 1/25 of one test dose must cause, a definite local reaction of the type known as the " positive Schick reaction."

The test is used to detect people who are susceptible to diphtheria and need immunisation, and to confirm immunity after immunisation. It involves the intradermal injection of a definite amount of diphtheria toxin known as the Schick dose ; a local skin reaction occurs in persons with less than a certain " level " of circulating antitoxin. The average amount of antitoxin necessary to ensure neutralisation of the test dose of toxin is 1/200 unit per c.c. of blood. There are, however, wide variations in the antitoxin content of the blood consistent with absence of reaction (the Schick negative state).

During epidemics, *e.g.*, in a school, the test will enable the physician in 24 to 48 hours to select the immune, who may continue school, while those giving a positive reaction are kept under special observation and examined bacteriologically. In cases of sore throat, where the diagnosis is in doubt, a negative reaction (if no antitoxin has been given) is strong evidence against clinical diphtheria.

The neutralisation of a toxin by the subject's antitoxin does not affect other substances present in the toxin which give rise to so-called pseudo reactions in certain sensitive people. It is therefore

SCHICK TEST FOR SUSCEPTIBILITY TO DIPHTHERIA

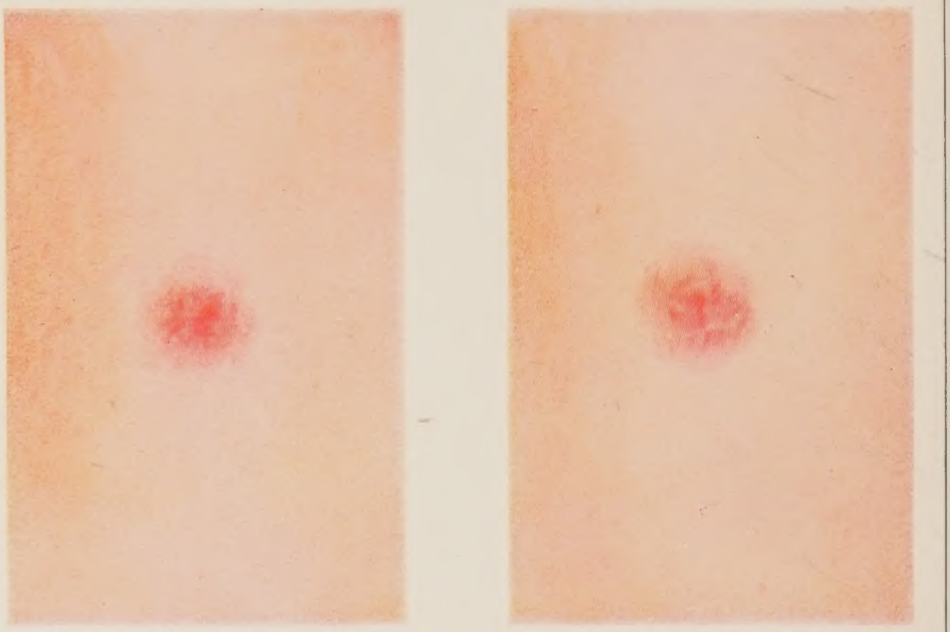
Control

NEGATIVE REACTION—IMMUNE

Control

POSITIVE REACTION—SUSCEPTIBLE

SCHICK TEST FOR SUSCEPTIBILITY TO DIPHTHERIA



Control

PSEUDO AND NEGATIVE—IMMUNE

Control

PSEUDO AND POSITIVE—SUSCEPTIBLE

necessary to use a control fluid in which the toxin has been destroyed by heat but the non-specific constituents remain unaltered.

TECHNIQUE AND READINGS

Into the flexor surface of the left forearm 0·2 c.c. of the toxin is injected intradermally *(see page* 6), into the right forearm 0·2 c.c. of the control. Two 1 c.c. all-glass syringes divided into tenths may be used, one for the toxin dilution and the other for the control. The needles must be short and stout and with short bevels. The reactions may be read at intervals of 24 to 48 hours, though five to seven-day readings are essential to detect late reactors and to reconsider earlier " doubtful " readings. By comparing the lesions on the two arms it is possible to decide how far a reaction is due to the active toxin and how far to non-specific constituents. (1) In *negative* reaction (immune) no reaction is visible on either arm. (2) In *negative-and-pseudo* reaction (immune), both arms show a similar flush which usually fades more rapidly than a true positive reaction. (3) In *positive* reaction (susceptible) the left arm shows a flush from 10 to 50 mm. in diameter; this fades and becomes brown, and a fine desquamation may occur. (4) In *positive-and-pseudo* combined reaction (susceptible), both arms show a flush, the left being larger, deeper and more persistent than the right. All " doubtful " reactions should be regarded as positive.

Pseudo-reactors are usually older children and adults because these have been exposed to diphtheria bacilli. They are often immune, since the exposures which have sensitised them have also led to the development of specific antitoxin.

As the staining of positive reactions may persist for weeks or months, many clinicians perform Schick tests on nurses and other women on the lower part of the thighs just above the patellæ.

N.B.—The readings of Schick Tests performed more than one hour after the administration of scarlet fever antitoxin (most commercial samples of which contain some diphtheria antitoxin) or of measles immune serum may be erroneous and should be disregarded if negative.

Schick Test Toxin is issued ready for use in a special diluent (borate buffer containing human serum with no detectable diphtheria antitoxin) which was devised at The Wellcome Physiological Research Laboratories to stabilise dilutions. The diluted toxin, if kept in a cold place, will be fully potent up to the date shown on the package. Although the diluted toxin contains antiseptic, clinicians are urged to use the whole of the contents at one session

if possible, or within a short period. It is highly undesirable that a small amount should be used and the remainder of the contents put aside for work some days or weeks later.

ACTIVE IMMUNISATION AGAINST DIPHTHERIA

Three types of prophylactics are issued, A.P.T., T.A.F. and T.A.M., all prepared from diphtheria toxin rendered harmless by formalin and heat *(i.e.*, Formol-Toxoid F.T.).

The injection is usually made intramuscularly or subcutaneously deeply into the arm, above the insertion of the deltoid. (It is wise to warn against active use of the arm for the remainder of the day, and if possible, to give the injection just before bedtime.) With all

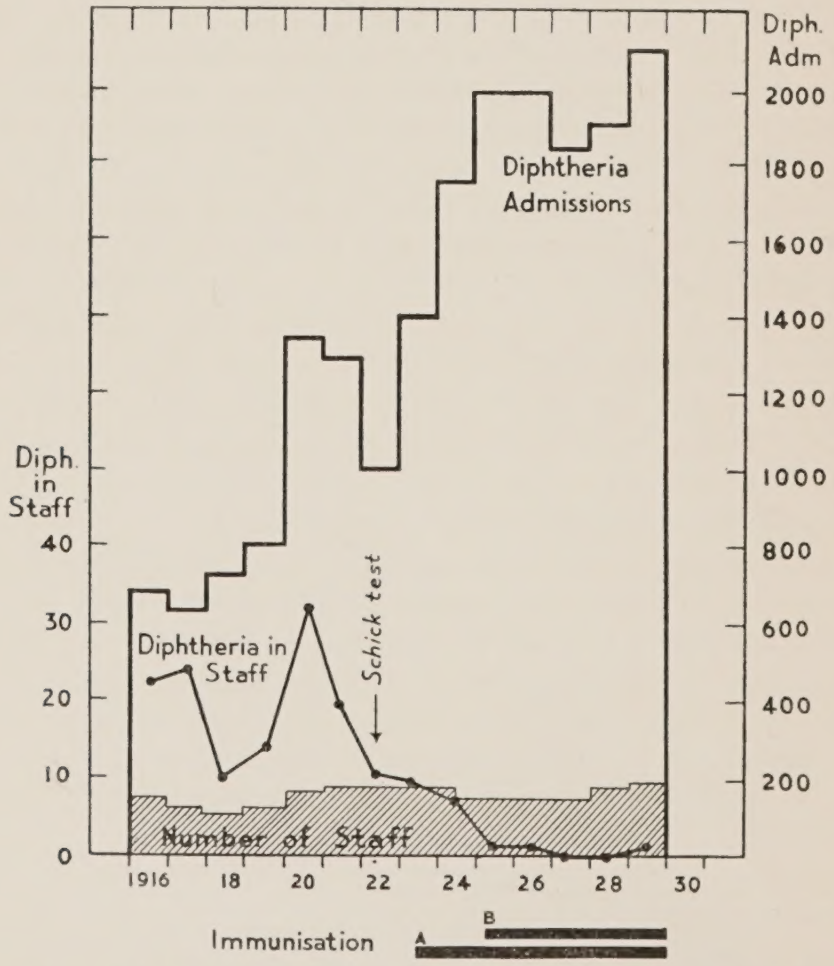


Diagram showing effect of immunising the staff of the Birmingham City Hospital against diphtheria. Columns represent diphtheria admissions, and curve the cases among the staff.

Inoculation of nurses began at A, and of domestic staff at B. Shaded area represents average strength of nursing and domestic staff each year. *(See* Harries, *Lancet*, 1939, I, 45.)

these prophylactics, 80 to 100 per cent of subjects will be found Schick-negative within six weeks of the final injection; the percentage varies in different groups, being higher amongst the Schick-positive children where the natural Schick-negative rate is high. Persons who are still Schick-positive after injections of prophylactic should receive further immunisation. A Schick-negative result after a course of prophylactic implies a useful level of circulating antitoxin, which gives almost complete protection against diphtheria and lasts, in all but a small percentage of people, for some years.

Diphtheria, almost invariably mild, has been reported from time to time in Schick negative reactors *(see note on page* 35). It is well, when signing "certificates" of immunised children, to include a warning that, notwithstanding immunisation, a "sore throat" should immediately be seen by a doctor; this course provides against error and misunderstanding, and safeguards the small percentage of "immunised" people whose immunity does not persist at a sufficiently high level.

Age.—Most city children less than nine months old are maternally immune and do not need immunisation, nor do they respond well to it.

Children between nine months and eight years old are mostly non-immune and rarely suffer from "reactions" after immunisation. Up to eight years one may therefore omit the Schick Test and immunise all these children. In older children it is wise to do a preliminary Schick Test.

Choice of Prophylactic.—This depends on *(a)* the amount of testing and supervision available, *(b)* the fear of "reactions," *(c)* the rapidity of immunity desired.

	Immunising Efficiency	Liability to Cause Reactions	Dosage
A.P.T.	Very high	Low, especially in young children	2 doses each of 0·5 c.c.; or 0·2 c.c. and 0·5 c.c. *(see page* 34)
T.A.F.	High	Very low at all ages	3 doses each of 1 c.c.
T.A.M.	Moderate	Low, especially in young children	,,
F.T.	High	Low only in young children	,,

The interval between the injections is of considerable importance, the best results being obtained when some weeks elapse between doses. When possible or convenient the interval should be four weeks, but three weeks is preferable to the shorter period of one week formerly used. When three doses are used the interval between second and third injections need not be longer than two weeks.

A.P.T. (ALUM PRECIPITATED TOXOID)

A suspension of the washed precipitate produced by the addition of alum to toxoid. The discovery that A.P.T. is a prophylactic of extraordinarily high immunising power in animals was made in The Wellcome Physiological Research Laboratories in 1926 ; its use in human preventive medicine was recommended in 1930.

This high immunising efficiency probably depends on the deposition of the relatively insoluble aluminium-toxoid complex at the site of injection and its gradual liberation. A small amount of local tissue reaction may occur—which may be an essential factor in the excellent immunisation which usually results—and a small painless nodule may form and gradually disappear. In most large collections of figures a very small number of local sterile " abscesses," healing rapidly after incision, have been noted.

Since A.P.T. was first prepared a number of improvements have been made to increase its antigenic efficiency while keeping the nitrogen and aluminium content low.

Some guide to the liability of individual patients to suffer from undue reaction during immunisation can be obtained from the response to the injection of the Schick control fluid. Patients who show a clear pseudo-reaction one or two days after the Schick Test will suffer from less reaction with T.A.F. than A.P.T.

Much has been claimed for the efficiency of one injection, but one dose of 0·5 c.c. of A.P.T. of proved high antigenic value may fail to give a high conversion rate unless the natural Schick-negative rate in the group is high, *e.g.*, after a recent epidemic in an institution. For general use two spaced doses give much better results, the conversion to the Schick-negative condition usually being well over 90 per cent. For children under eight a first dose of 0·5 c.c. which very rarely causes troublesome reactions may be used ; a second dose of 0·5 c.c. is given four weeks later. For older children and adults a first dose of 0·2 c.c. has been widely used. This " detects " an unusually sensitive person who may show some local reaction.

For these subjects the further immunisation may be a second dose of 0·2 c.c. of A.P.T. four weeks later or two further doses of 1 c.c. of T.A.F. at intervals of three to four weeks. For those showing no reaction after the first injection a second dose of 0·5 c.c. may be given four weeks later.

A.P.T. is usually injected intramuscularly.

T.A.F. (TOXOID-ANTITOXIN FLOCCULES)

A suspension of the floccules formed when toxoid and antitoxin are mixed in appropriate neutralising amounts. This is the least likely of all prophylactics to cause reaction and is much used for the immunisation of adults.

T.A.M. (TOXOID-ANTITOXIN MIXTURE)

The toxoid is diluted and partially neutralised by antitoxin. This is a less efficient prophylactic than any of the others and is less used than formerly.

F.T. (FORMOL-TOXOID)

Is still much used on the Continent (Ramon's anatoxine) and in Canada. It is diphtheria toxin detoxicated by the action of formalin and heat, and is the preparation from which the other three prophylactics are made. It has high immunising efficiency, but unfortunately tends to cause severe reactions particularly in older subjects, and has been largely replaced in this country by T.A.F. for adults and A.P.T. for children.

RE-INOCULATION OF PROPHYLACTIC

Some individuals who have once been Schick-negative relapse to the positive state and have little or no circulating antitoxin in their blood ; thus it may happen that in an institution trouble may arise from cases of infection, generally mild, in subjects previously immunised. When circumstances indicate that a more durable immunity is needed this can be achieved by periodic reinjection of diphtheria prophylactic. Thus, children who have been immunised in infancy with two doses of A.P.T. might receive a third injection of prophylactic just prior to the beginning of school life, and a fourth at the age of eight or nine years. Alternatively a dose might be given if there is evidence that a severe wave of infection is occurring locally.

Some experienced workers recommend the injection of one dose of prophylactic into all natural Schick-negative reactors at the time of reading in order to raise the level of circulating antitoxin.

COMBINED IMMUNISATION

Some workers give scarlet fever prophylactic and diphtheria prophylactic together, making the mixture in the syringe immediately before injection.

Diphtheria Prophylactic T.A.F. may be given in conjunction with tetanus toxoid *(page* 41). Two doses of 1 c.c. Tetanus Toxoid and 1 c.c. T.A.F. mixed in the syringe are injected at an interval of four to six weeks, and this should be supplemented by a third dose of 1 c.c. of T.A.F. after a further two weeks. The supplementary dose of T.A.F. may be given midway between the two injections of the combined prophylactic, but the results are not so satisfactory. The results now available indicate that titres of circulating antitoxin are at least as good after these combined antigens as after either antigen given separately.

A.P.T. and whooping cough vaccine *(page* 50) is a useful combination for children.

Combined diphtheria prophylactic and T.A.B. vaccine *(page* 44) is probably contra-indicated for adults as some persons would have very severe reactions.

For combined tetanus toxoid and T.A.B. vaccine *(see page* 42).

Storage.—The optimum temperature of storage of diphtheria prophylactics is just above freezing point, i.e., 2° to 4° C. It is important that they should not be frozen solid, since deterioration may then be rapid.

When stored in a cool, dark cupboard, prophylactics retain their potency very well. Except in an emergency, however, they should not be used after the date on the label.

The entire contents of ampoules should be used at one sitting.

THE DICK TEST AND ACTIVE IMMUNISATION AGAINST SCARLET FEVER

DICK TEST

The Dick Test indicates whether individuals are susceptible to or immune from scarlet fever. In the susceptible, immunity can be produced by the injection of prophylactic.

The toxin, which is the filtrate of a culture of hæmolytic streptococcus, is issued in diluted form ready for the test. It should be used within the period indicated by the advance date.

Heated toxin is supplied for use as a control.

TECHNIQUE AND READINGS

A syringe as well as the needle should be rinsed out with skin test solution before use. The test consists of injecting intradermally

into the left forearm 0·2 c.c. of the diluted toxin. Another syringe and needle are taken and 0·2 c.c. of the same diluted toxin, which has been steamed for four hours, is injected into the right forearm as a control. The reaction which appears in from four to sixteen hours, and may have begun to disappear at the time of the twenty-four hour reading, may be *positive, negative, or negative-and-pseudo*, as in Schick testing *(see page* 30). Theoretically a *positive-and-pseudo* reaction should occur, but it is almost unknown. Experience indicates that almost all people who give a completely negative reaction are immune to scarlet fever. Patients who are not immune show a red flush at the site of injection. The slightest reddening or flush, no matter how faint, is read as positive if it measures as much as 10 mm. in diameter. The colour may be blanched by stretching the skin. The toxin issued causes pseudo-reactions in only a small proportion of children, and many workers after a short experience dispense with the control. With reference to the reading of the Dick Test, G. F. and G. H. Dick (Monograph on " Scarlet Fever," 1938, *page* 80) state that " . . . the commonest error of all is interpreting positive reactions as negative. The tendency to regard slightly or even moderately positive reactions as negative is due to familiarity with the Schick Test, which is usually interpreted as negative unless there is induration. Skin reactions with scarlet fever toxin are never indurated. They should be observed in a bright light between 20 and 24 hours after the injection. Observations made earlier than 18 hours or after 24 hours are not reliable."

About 20 per cent of children are immune at the age of two, whereas 70 per cent or more of adults are immune.

Some 80 per cent of patients suffering from definite scarlet fever will give a positive response to the Dick Test during the first two days of the attack. Those with a very vivid universal rash may not show any greater reddening at the site of the injection, probably because the capillaries are already dilated to their extreme extent. As in these cases the diagnosis is usually very easy to make, the Dick Test is rarely required here.

The results obtained in various hospitals demonstrate that by testing the nurses, and where necessary actively immunising, scarlet fever amongst them is reduced to the point of disappearance. The immunisation of children and others liable to be exposed to infection is sometimes carried out. Unfortunately the large number of injections of prophylactic required to produce immunity is a deterrent to mass immunisation. Some writers advise the immunisation of family contacts immediately after the infected child

has been removed to hospital, believing that the contacts may become immune before the convalescent child returns from hospital. In an epidemic at a school or institution the " Dick negative reactors" can be regarded as immune to scarlet fever.

ACTIVE IMMUNISATION AGAINST SCARLET FEVER

The immunising course consists of four or five weekly subcutaneous or intramuscular injections of a scarlet fever toxin which contains no blood or serum. The Dicks recommend superficial subcutaneous injections given " near enough the under surface of the skin to produce a visible subcutaneous lump." They claim that this technique is helpful in avoiding both severe local and severe general reactions.

Dosage is cautious as it is toxin which is used. If necessary, doses which cause no reaction may be repeated after an interval of a few days, provided that an interval of at least two to three weeks occurs between the last two doses of the course.

Most workers are agreed that a first injection of 500 or even 1000 doses rarely causes a reaction (except in those with a vivid positive Dick response). There is no general agreement as to the best subsequent dosage. The tendency in this country is to give a total of 80,000 to 100,000 skin test doses in four or five injections. Sequence I represents the average practice of those who favour large doses and expect high immunity at the cost of a certain percentage of " reactions." It has been stated that the inoculation of 0·2 c.c. of adrenaline solution in each dose materially reduces the incidence of reactions in those prone to them.

SCARLET FEVER PROPHYLACTIC

Toxin is issued in two strengths :—

A. Containing 2500 skin test doses per c.c.
D. Containing 50,000 skin test doses per c.c.

Sequence I

500	= 0·2 c.c.	Strength A
2000	= 0·8 c.c.	„ A
5000	= 0·1 c.c.	„ D
25,000	= 0·5 c.c.	„ D
50,000	= 1·0 c.c.	„ D

Some physicians would choose a shorter sequence such as

Sequence II

1000	= 0·4 c.c.	Strength A
5000	= 0·1 c.c.	„ D
and 10,000	= 0·2 c.c.	„ D
or 20,000	= 0·4 c.c.	„ D

A good working compromise, particularly in residential schools, would consist in giving the first three (or perhaps four) injections of Sequence I and Dick testing one to two weeks later; those children still positive would continue to receive injections.

The Dicks recommend the following—

Sequence III

650=0·3 c.c. (approx.) Strength A
2500=1·0 c.c. „ A
10,000=0·2 c.c. „ D
30,000=0·6 c.c. „ D
100,000–120,000=2·0 c.c. to 2·4 c.c. Strength D

They suggest an injection of one-quarter or one-half the usual first dose as a preliminary in persons who are highly susceptible as shown by large bright skin reactions.

The majority of those inoculated become negative within a few weeks but it is not known with certainty how long the immunity will last. It is probably wise to Dick Test those specially exposed, *e.g.*, nurses, every six or twelve months and to give injections of prophylactic to those who are positive.

If an excessive dose of prophylactic is given in the earlier stages of immunisation the patient may show what has been called " the scarlatinoid syndrome," namely a generalised rash with malaise or vomiting. Such symptoms are very rarely observed as a result of the usual course of immunisation; they disappear in 24 to 48 hours, and need not cause undue alarm. It is probable that the patient will become more rapidly immune if the dose has been large enough to cause this syndrome.

N.B.—Syringes and needles used for injection of scarlet fever toxin should be sterilised by dry heat, in an autoclave, or by boiling in distilled water. Alcohol or other disinfectants which may precipitate the toxin should be avoided.

THE SCHULTZ-CHARLTON BLANCHING TEST

To perform this test 0·2 c.c. of a suitable dilution of scarlet fever antitoxin is injected into the skin of a scarlet fever patient in whom a uniform rash not more than 60 hours old is present. When the test is positive an area of local blanching from 20 mm. to 50 mm. in diameter usually appears within 8 to 24 hours and lasts from 12 to 48 hours. A positive reaction occurs in many patients tested during the early stage of the rash.

The Schultz-Charlton and Dick Tests will often serve to clear up an obscure case of fever with an atypical rash. A positive blanching result probably justifies a diagnosis of scarlet fever whatever the reading of the Dick Test. If the Dick and Schultz-Charlton Tests are negative this is adverse evidence but does not completely exclude a diagnosis of scarlet fever.

Attention is also directed to the use of the pharyngeal swab in the elucidation of the doubtful case. Absence of hæmolytic streptococci on *good* blood agar is strong evidence against a diagnosis of scarlet fever.

Stability.—With regard to the stability of Dick Test dilution, prophylactic for active immunisation and Schultz-Charlton Test solution, good results have been obtained with material which has been kept in the ice-chest for several months. Except in an emergency, however, these materials should not be used after the date on the label.

ACTIVE IMMUNISATION AGAINST STAPHYLOCOCCUS INFECTIONS

Immunisation with staphylococcus toxoid has been used as a combined method of prophylaxis and treatment of chronic infections. The antigen used is a toxic filtrate rendered harmless, as in the production of diphtheria toxoid, by formalin and heat. In Melbourne and Toronto highly promising results in a considerable number of cases have been reported in conditions such as recurring boils and carbuncles and chronic staphylococcal skin infections; experience suggests that long-standing typical sycosis and uncomplicated acne may not respond to treatment with toxoid. In England the general results agree with those reported elsewhere, although there is still some difference of opinion among dermatologists concerning the efficiency of this form of treatment.

There are on record a few instances of puzzling relapses in patients who showed rapid disappearance of long-standing symptoms after the use of toxoid.

Two preparations are issued:—

Staphylococcus Toxoid A (1/10 dilution) and
Staphylococcus Toxoid B (undiluted Toxoid)

Dosage.—It is wise to start with 0·5 c.c. of the 1/10 dilution, Staphylococcus Toxoid A, given intramuscularly. If a negligible local reaction occurs, 0·1 c.c., 0·2 c.c., 0·3 c.c., etc., of the undiluted

toxoid, Staphylococcus Toxoid B, may be given at intervals of four or seven days, the dose being progressively increased until eventually about 1 c.c. is being administered.

In the event of an undue local or general reaction occurring after one of these doses, the next injection of the series should be the same amount or less.

Some clinicians combine staphylococcus toxoid and vaccine.

N.B.—Commence with Toxoid A; Toxoid B is ten times stronger.

ACTIVE IMMUNISATION AGAINST TETANUS

The toxin of *Clostridium tetani*, rendered atoxic by formalin, *i.e.*, tetanus toxoid, can be used to produce a high immunity in those persons who may be exposed to the danger of tetanus, *e.g.*, soldiers, agricultural workers, gardeners and A.R.P. workers. In certain tropical regions, in which umbilical tetanus of infants is common, the disease has been prevented by active immunisation of mothers during pregnancy.

Dosage.—The usual method is to inject two doses of 1 c.c. intramuscularly into the left arm. A rather long interval between injections, *e.g.*, six weeks, is favourable to the production of high immunity. Longer intervals than six weeks give, if anything, better antitoxic response, but are usually inconvenient administratively.

Some antitoxin may appear before the second injection, but it rises rapidly during the second week after the second injection to a level which evidence suggests is ample to give a high degree of protection against the risk of tetanus following infected wounds, abrasions, etc. A third injection of 1 c.c. is sometimes given after a long interval, *e.g.*, one year, and almost invariably produces a dramatic increase in circulating antitoxin.

In recent investigations at The Wellcome Physiological Research Laboratories it was found that women gave significantly higher titres than men.

The active immunity induced by the toxoid is long-lasting and even when the concentration of antitoxin in the blood is becoming somewhat reduced in the course of time, the patient possesses an active " potential immunity," so that if infected with tetanus the antitoxin content of the blood will rise very rapidly.

Reactions after the injection of tetanus toxoid are rare and usually mild. Some are anaphylactic in type and are mostly

observed after the second injection. Patients with a history of asthma or hay fever, etc., may be more liable than others to these allergic manifestations. It is suggested, therefore, that a reduced dose, for example, 0·1 c.c., should be administered to them, followed, after some hours or the next day, by the ordinary dose of 1 c.c. if no symptoms have occurred.

The injections of 0·5 c.c. of 1:1000 adrenaline subcutaneously, repeated if necessary, controls the allergic reaction.

The previous administration of tetanus antitoxin should not be taken as a contra-indication to the use of tetanus toxoid.

ACTIVE IMMUNISATION AGAINST TETANUS AND ENTERIC FEVER

Active immunisation with combined tetanus toxoid and anti-typhoid-paratyphoid vaccine has been recognised as an effective method of prophylaxis against tetanus and the enteric fevers.

The efficiency of anti-typhoid-paratyphoid vaccine in prophylaxis is the best established of all facts concerning the use of bacterial vaccines. As a result of anti-typhoid inoculation and other measures, typhoid fever has become comparatively rare and unimportant.

The immunisation against tetanus and the enteric fevers by the administration of two doses of the combined antigen is of great practical importance, and recent investigations have shown that when tetanus toxoid is administered in combination with T.A.B. vaccine the antitoxic response may be much greater (up to five times as great) than when the toxoid is given alone.

Anti - typhoid - paratyphoid vaccine with tetanus toxoid (T.A.B.T.) is issued in sets of two doses as follows :—

First Dose (No. 1).—1 c.c. tetanus toxoid containing 500 million *Bact. typhosum* ; 375 million *Bact. paratyphosum A* ; and 375 million *Bact. paratyphosum B.*

Second Dose (No. 2).—1 c.c. tetanus toxoid containing 1000 million *Bact. typhosum* ; 750 million *Bact. paratyphosum A* ; and 750 million *Bact. paratyphosum B.*

The usual method of immunisation is to inject two doses of 1 c.c. (one dose of each strength) deeply into the subcutaneous tissues of the left arm. At least one month should elapse between the first and second doses. For those continually exposed to

infection a dose of T.A.B.T. may be given each year to ensure a continued high level of immunity.

Such reactions as may occur after the injection of T.A.B.T. are rarely greater than those following the administration of T.A.B. Anaphylactic reactions which may occur due to the tetanus toxoid constituent of the prophylactic are rare and usually mild. Patients who give a history of asthma, hay fever, etc., may be more liable than others to these allergic manifestations. An injection of 0·5 c.c. of 1 : 1000 adrenaline subcutaneously, repeated if necessary, will control the symptoms.

ACTIVE IMMUNISATION AGAINST VIRUS DISEASES

A description of methods of prophylaxis used so successfully in smallpox and rabies is outside the scope of this book. Much of the work on other virus diseases such as influenza and poliomyelitis is still in the experimental stage. In yellow fever an attenuated virus grown in tissue culture or chick embryo is an efficient immunising agent *(see page* 15). In veterinary practice, active immunity to dog-distemper is induced by the use of either (1) " vaccine " (formolised tissues of infected dogs), followed 14 days later by " virus " (tissues of infected ferrets); or (2) " virus " followed one and a half or two hours later by immune serum (obtained from dogs injected with distemper virus).

BACTERIAL VACCINES

A bacterial vaccine of the class here considered may be defined as a sterilised and standardised emulsion of a micro-organism. It is usually a broth culture or a suspension in 0·7 per cent saline of a culture made on agar or other solid medium. This is sterilised by heating to a temperature just sufficient to kill the organisms, and subsequently standardised according to the number of micro-organisms present in 1 c.c. The vaccines are submitted to bacteriological tests for purity, and phenol or formalin is added as a preservative.

Bacterial vaccines are administered by subcutaneous injection *(page* 6), and act by producing an active immunity to the corresponding living organisms. The cells of the body are stimulated by the introduction of the inoculum to form specific antibodies. This production of antibodies goes on for a considerable time after inoculation and the resistance thus acquired lasts longer than the passive immunity due to the injection of immune sera *(see page* 4). Unlike the latter, however, vaccines do not confer immediate protection. Although attention was formerly directed to the production of a " negative phase " following the injection of varying quantities of vaccine, it is now generally accepted that this is of no practical importance.

In some cases it may be safe to adopt routine methods in using vaccines ; in acne, for instance, doses of 100–1000 million staphylococci may be given every 10 days, and " stock vaccines " may be used. In other cases, the effect of each dose of vaccine must be carefully noted and the treatment must proceed with great caution. It is also thought better, in some instances, to prepare " autogenous vaccines " from the micro-organisms isolated from the patient.

In addition to the enteric group, the diseases for which bacterial vaccines have some reputation are those described as chronic localised infections, such as acne (staphylococcus and acne bacillus) ; boils, carbuncles, etc. (staphylococcus) ; cystitis, pyelitis, etc. *(Bact. coli)* ; gonorrhœa, prostatitis, etc. (gonococcus) ; abscesses, septicæmia, endocarditis, etc. (streptococcus).*

ANTI-TYPHOID-PARATYPHOID VACCINE (T.A.B.)

The value of T.A.B. vaccine was amply confirmed by the experiences of the military medical services of all the Great Powers

* Attention is called to the promising results obtained in the treatment of streptococcal, pneumococcal, gonococcal, meningococcal, *Brucella* and *Bact. coli* infections by drugs of the sulphanilamide group. *See special literature, sent an request.*

of Europe during the war of 1914–1918. As a result of anti-typhoid inoculation, typhoid fever, which was formerly one of the commonest and most fatal diseases among armies in time of war, has now become comparatively rare and unimportant. The addition of paratyphoid strains to the vaccine protects against infection with these organisms also.

T.A.B. vaccine contains 1000 million *Bacterium typhosum* and 750 million each of *Bacterium paratyphosum A* and *B* in each c.c., and is prepared from smooth virulent strains.

Either two doses of 0·5 c.c. and 1 c.c., or three doses of 0·1 c.c., 0·5 c.c. and 0·75 c.c. are usually given subcutaneously with the usual aseptic precautions. The former dosage has been recommended by the Army authorities ; the latter has been much used in civilian practice and is associated with somewhat less severe reactions. The interval between injections should be at least seven days, and preferably longer.

There is no hard and fast rule about dosage and the doctor may modify the usual scheme of injections for adults in accordance with body-weight and physical condition, and with the reactions he observes after earlier doses of the series. In general, women receive smaller doses than men. In the presence of an epidemic a first dose of 0·1 c.c. has been specially recommended.

For those frequently exposed it is advisable to re-vaccinate with one or two doses every one to two years.

Following the administration of the vaccine, local symptoms, consisting of redness, swelling, pain and tenderness at the site of injection, may begin to appear in two or three hours. Constitutional symptoms such as malaise, nausea, headache, etc., may also occur but usually disappear within 36 hours. If they are marked, the patient should be kept in bed for a day after the injection. If the vaccine be given late in the afternoon and no exercise taken afterwards, the chances of reaction are diminished. If a reaction does occur it will happen during the night and be less likely to upset the patient. Alcohol in any form should be forbidden for at least 24 hours after vaccination.

Fear of the production of a " negative phase " with lowered resistance has sometimes prevented the use of enteric vaccine during an epidemic. It has, however, been used in numerous instances during actual epidemics and has been given to nurses in attendance on enteric fever cases without any evidence of lowered resistance resulting.

Young children tolerate injections of T.A.B. vaccine better than adults. The dosage is necessarily arbitrary. When children aged 18 months have received two or three doses each of 0·1 c.c., reactions have been negligible. Children aged 5 years have been given two doses of 0·1 c.c. to 0·2 c.c., followed by 0·2 c.c. to 0·4 c.c., or three doses of 0·1 c.c., 0·2 c.c. and 0·3 c.c. respectively.

Although oral administration of T.A.B. vaccine is more convenient than the subcutaneous method it confers uncertain protection and is therefore seldom justifiable.

Anti-typhoid vaccine is no longer recommended as a curative agent in typhoid fever.

For active immunisation against Tetanus and Enteric Fever with a " combined " prophylactic, *see page* 42.

ANTI-TYPHOID-PARATYPHOID A, B AND C VACCINE (T.P3)

This vaccine, prepared from smooth virulent strains, contains 1000 million *Bacterium typhosum*, and 750 million each of *Bacterium paratyphosum A*, *B* and *C* in each c.c. Most of the remarks under T.A.B. Vaccine also apply to this vaccine.

ANTI-TYPHOID-PARATYPHOID A AND B AND CHOLERA VACCINE

This vaccine is prepared from *Vibrio choleræ* in addition to typhoid and paratyphoid strains, the doses and intervals being similar to those mentioned above.

The Ministry of Health has directed that the following names are to be used as " proper " names.

(1) Anti-typhoid-paratyphoid A, B and C Vaccine.

(2) Anti-typhoid-paratyphoid A and B and Cholera Vaccine.

No abbreviation should be made in respect of the latter name.

V. CHOLERÆ VACCINE

This vaccine, consisting of killed cultures of the *Vibrio choleræ*, is intended for prophylactic use and not as a curative agent. Each c.c. contains 10,000 million organisms. A first dose of 0·5 c.c. followed by a second of 1 c.c. a week later is suggested as representing the average dosage used in recent years.

Although no large groups of figures comparable with those obtained in the early well-controlled work with typhoid-paratyphoid vaccine are available, anti-cholera vaccination was considered so satisfactory that it was used as a routine in several of the armies in the Eastern campaigns during the war of 1914–1918. Accumulating experience has confirmed the earlier expectations of its value in combating epidemics of cholera.

ACNE VACCINES, MIXED

These are for use in ordinary cases of acne usually characterised by the presence of comedones and pustules. A bacteriological examination of such cases shows an infection by the acne bacillus with or without staphylococcus *(aureus, albus* or *citreus)*. The initial dose is usually four to five million acne bacilli. Subsequent dosage is regulated by the local effect. Some clinicians begin with much larger doses, such as 50 to 100 million.

In the pustular and furuncular forms of acne without comedones, Staphylococcus Vaccine, Mixed, is used *(see also* Staphylococcus Toxoid, *page* 40).

B. COLI VACCINE

This vaccine contains the *Bacterium coli commune* and may be used in coliform infections of the bladder, ureters, kidneys and peritoneum, in appendicitis due to the *Bacterium coli*, in mucous and ulcerative colitis, and in *Bacterium coli* infection of the uterus and gall-bladder.

The initial dose is 5 to 15 million organisms, which may be repeated or increased, according to the reaction produced, from two to ten days later. Some clinicians advocate much higher doses.

CORYZA VACCINE

Research work by Dochez and others appears to have proved that a filterable virus is the infectious agent of the common cold. No vaccine against the " virus " cold is yet available.

The secondary symptoms of a " cold " are usually attributed to infection with such organisms as *Corynebacterium hofmanni*, *Streptococcus pneumoniæ*, *Neisseria catarrhalis*, *Bacterium friedländeri*, *Hæmophilus influenzæ*, staphylococcus and streptococcus. Coryza vaccine is prepared from these organisms. Discouraging results have been obtained in field trials in man when regard has been paid to statistical principles, but many clinicians are satisfied with the results obtained in certain individuals.

The doses used vary in the hands of different practitioners. For prophylactic use, 0·5 c.c. may be used as an initial dose. According to the reaction this dose may be increased to 1 c.c. or even 1·5 c.c. after an interval of a week.

GONOCOCCUS VACCINE

This vaccine, prepared from the *Neisseria gonorrhœæ* (gonococcus), has been used in the chronic and later stages of gonorrhœa, in gonorrhœal prostatitis and in such generalised infections as

gonorrhœal arthritis. It has been used in cases of chronic gonococcal iritis but caution is necessary in using it in this condition. Very small doses, *e.g.* 0·5 to 1 million cocci have been proposed.

The initial dose given by different authorities varies considerably, being, according to some, half a million. Subsequent dosage is regulated in accordance with the constitutional effect.

INFLUENZA VACCINE, MIXED

This vaccine contains *Hæmophilus influenzæ* (400 million), pneumococci (200 million) and streptococci (80 million). Influenza is caused by a filterable virus and experiments are now being made to produce immunity in man by means of a virus vaccine. Mixed influenza vaccine may be injected to give protection against the "secondary invaders" causing pulmonary complications. The dose suggested is 0·5 c.c. followed after an interval of 10 days by 1 c.c., but the results are no better than with coryza vaccine.

BR. MELITENSIS VACCINE (MEDITERRANEAN FEVER)

The vaccine may be used in cases of infection by the *Brucella melitensis* or *Br. abortus*. In the most acute cases with high fever and severe intoxication it should not be used.

Although success has been claimed in some cases the results of vaccine treatment are generally unsatisfactory. A first dose of 100 million organisms may be given and repeated in a week or 10 days according to the indications of the case.

PNEUMOCOCCUS VACCINE

This vaccine contains various strains of the main types of the *Streptococcus pneumoniæ* (pneumococcus). It has been used in infections such as pneumonia, empyema, pericarditis, endocarditis, septicæmia, meningitis and pneumococcal arthritis, in which the causative organism is the pneumococcus. The usual dose is 10 to 50 million organisms, which may be repeated, according to the reaction produced, every two, five or ten days.

M. RHEUMATICUS VACCINE

This vaccine contains several strains of streptococci *(Micrococcus rheumaticus* of Poynton and Payne) obtained from cases of acute rheumatism. It may be used in cases of persistent relapsing rheumatism. The dose is 10 million organisms, gradually increasing to 50 million, administered at intervals of seven to ten days.

STAPHYLOCOCCUS AUREUS VACCINE

This vaccine contains only *Staphylococcus pyogenes aureus* and is usually given in doses similar to those of staphylococcus vaccine, mixed *(see below and also refer to* staphylococcus toxoid, *page* 40).

STAPHYLOCOCCUS VACCINE, MIXED

This vaccine, which contains *Staphylococcus pyogenes aureus, citreus* and *albus* may be employed in various staphylococcal infections, such as pustular acne, furunculosis, carbuncle, sycosis, blepharitis and localised abscesses. The initial dose is usually 200 million organisms. A second dose of the same number may be given in a week's time; if the first dose has produced only slight and evanescent constitutional effects 1000 million organisms may be necessary. Many authorities recommend much smaller doses.

STREPTOCOCCUS VACCINE, POLYVALENT

This vaccine contains many strains of streptococci obtained from cases of erysipelas, scarlet, puerperal and rheumatic fevers, septicæmia, angina, pneumonia, ulcerative endocarditis and pyorrhœa alveolaris. It has been used in all forms of localised or generalised streptococcal infection, *e.g.* abscesses, septicæmia, pyæmia, otitis media, endocarditis, peritonitis of streptococcal origin, puerperal septicæmia and erysipelas. The dose varies from 10 to 50 million organisms at intervals of from one to three weeks according to the reaction produced.

WHOOPING COUGH VACCINE

This vaccine is prepared from recent strains of *Hæmophilus pertussis* (the Bordet-Gengou bacillus) isolated by the cough-plate method, and in the smooth virulent phase 1. The cultures are grown on Bordet-Gengou medium and are killed by antiseptic. Only one strength is issued, viz. 10,000 million organisms per c.c. There is no satisfactory laboratory test for potency nor does the clinical evidence yet provide complete proof that all the batches of vaccine made in different parts of the world are effective in prophylaxis. A properly prepared phase 1 vaccine offers at present the only hope of protection and appears to have produced considerable immunity according to some of the published clinical trials. Its extended use would therefore seem desirable in view of the present incidence of whooping cough and the increased possibility of infection under war-time conditions. There is no general agreement that vaccine is effective in treatment.

Since whooping cough is most serious during the early years of life it is advisable to immunise during infancy, preferably commencing the course at the end of the second or third months. It is

generally considered that a period of several months must elapse before the resulting immunity is complete but some observers believe that a useful degree of immunity can be obtained in a much shorter period.

Authorities differ over the dosage for prophylaxis. The following scheme is less tedious than some which have been recommended, although it entails five injections. These are given subcutaneously. The first dose is 1 c.c. given over the left biceps ; the second, 1·5 c.c. over the right triceps ; the third, 1·5 c.c. over the left triceps ; the fourth, 3 c.c. (1·5 c.c. over each deltoid). Total=7 c.c. (equivalent to 70,000 million organisms). The injections may conveniently be given at intervals of one week. Reactions appear to be somewhat more troublesome than in anti-diphtheria immunisation, especially in older children.

Recent investigations suggest that much smaller doses, *e.g.* 0·5 c.c. or 5000 million organisms, afford satisfactory protection if the interval before the final injection of the series is four weeks. Three doses may be given at intervals of three to seven days, followed by a fourth dose after one month. Total=2 c.c. (equivalent to 20,000 million organisms).

Re-stimulation after a year or so, for example, with a single injection of 0·5 c.c., may induce a marked "secondary stimulus response " and a high level of immunity. It is also suggested that children who have received a course of vaccine and are exposed to infection should be given at least one further dose of vaccine to reinforce their immunity.

If immunisation is delayed till after the sixth month, some of the doses of Whooping Cough Vaccine may be combined with Diphtheria Prophylactic A.P.T. or T.A.F. and given from the same syringe in order to confer immunity against Pertussis and Diphtheria at the same time. It is desirable that all young children should be adequately protected against both diseases.

There seems little evidence justifying the use of vaccine for treatment but some clinicians recommend six subcutaneous injections, each ranging from 100 million to 1000 million organisms, administered on alternate days. Others suggest 1000 million to 3000 million for older children, increasing to 5000 million or more organisms in six injections ; smaller doses are stated by these workers to be less effective.

Storage.—Vaccines like other biological products should be kept in a cool, dark place. Storage below freezing point is harmful. The optimum temperature is just above freezing point, *i.e.*, 2° to 4° C. Except in an emergency they should not be used after the date on the label.

TUBERCULINS

Tuberculin was originally prepared by Koch by growing the tubercle bacillus in a 5 per cent glycerol-broth medium for 6 to 8 weeks at 38° C., the mature culture being sterilised in a water bath at 100° C., evaporated to one-tenth of the original volume and filtered. The filtrate, which is a clear, brown, viscous fluid containing about 40 per cent of glycerol, is known as tuberculin.

Evidence of laboratory workers and clinicians indicates that there is little justification for the use of P.T.O., T.O.A., B.E., T.R., etc., as these produce similar effects to those caused by Koch's Old Tuberculin (T.=human or P.T.=bovine), but are less potent. There is no generally accepted clinical evidence that anything would be lost if Old Tuberculin (T.) were adopted to the exclusion of all other crude tuberculins; the commonly accepted methods of standardisation indicate that T. and P.T. are indistinguishable. The activity of the various preparations, human and bovine, depends on a single protein constituent (Purified Protein Derivative or P.P.D.) *(page* 53).

T.O.A. and P.T.O. contain one-tenth of the amount of active "tuberculin" in Old Tuberculin (T.); B.E. about one-twentieth, T.R. about one-hundredth. In changing from the use of any of these materials to T. the dilution should be correspondingly reduced.

Tuberculin is tested for potency by injecting corresponding dilutions of the tuberculin under investigation and the official International Standard Tuberculin into tuberculous guinea-pigs and comparing the specific intradermal reactions which develop.

Diagnostic Use of Tuberculin (T.).—Old Tuberculin (T.) may be used for diagnostic purposes by the intradermal (Mantoux and Moussu), dermal (von Pirquet), percutaneous or subcutaneous method.

In the **Mantoux Test** the usual first dose recommended where there is a possibility of active tuberculosis is 0·1 c.c. of a dilution of 1 : 10,000. It has been stated that if this amount gives a negative reaction it is unnecessary to inject 0·1 c.c. of 1 : 1000 and one may safely proceed to 0·1 c.c. of 1 : 100; the patient is regarded as negative only if this dose causes no reaction. Other investigators have used 0·1 c.c. of 1 : 1000 as the initial routine dose and it is agreed that the stronger the dilution of tuberculin used the more

positives will be obtained. If it is desired to use a control a simple dilution of the uninoculated culture fluid which has been concentrated may be employed. The work of D'Arcy Hart suggests that one may dispense with the control.

Interpretation of Tuberculin Reaction.—A positive reaction consists of erythema surrounding an area of œdema or occasionally œdema without the erythema. As there is some difference of opinion about the criterion of a positive reaction and the times of reading the recommendations of various authorities are now given. D'Arcy Hart (1932) accepts as positive a reaction with a diameter of 5 mm. taking the 48 hours' reading, but many workers insist on a minimal diameter of 10 mm. Aronson (1934) also finds it best to read at 48 hours. Kayne (1936) defines a positive reading as follows :—At 48 hours : area of erythema with or without swelling 10 mm. in diameter (mean) ; at 72 hours : area of erythema *and* swelling 7 mm. in diameter (mean). He also considers that the preferable time for reading is at 72 hours, on account of the occurrence of non-specific reactions at 24 hours, disappearing at 48 hours. Madsen and Holm (1935) also read at 72 hours instead of at 48 hours.

The main *clinical value* of the test is that a positive reaction in early life will strongly indicate active tuberculosis, while a negative reaction in later life (except in very advanced tuberculosis) may be very helpful to the clinician in indicating the absence of an existing or past tubercular infection. D'Arcy Hart's results obtained from approximately 1700 patients, both clinically tuberculous and non-tuberculous, show that the percentage of clinically tuberculous who give a positive Mantoux reaction at all ages is above 90 per cent ; the clinically non-tuberculous give a positive reaction in only 20 per cent up to the age of 5 and approximately 30 per cent up to the age of 10. By the age of 20 more than half the community have suffered from some past tubercular infection, and while showing no clinical signs, have apparently overcome the attack successfully. Their sensitivity is indicated by a positive Mantoux Test.

In the **von Pirquet Test,** a minute drop of undiluted Tuberculin T. is placed on the skin and a small superficial scarification made through the drop of tuberculin. A similar scratch may be made with a clean instrument through a drop of control fluid. A positive reaction is indicated by a papular swelling with a hyperæmic area 12 to 25 mm. in diameter at the site of the scarification. It appears in 24 hours and gradually fades into a small pigmented area.

Tests with various **ointments**, analogous to Moro's Percutaneous Test, have been introduced from time to time, but have the disadvantage that one cannot ensure that the same amount of tuberculin is absorbed in successive tests.

The **subcutaneous test of Koch** is no longer regarded as safe because severe reactions may sometimes occur.

Therapeutic Use of Tuberculin (T.).—Some workers still believe that treatment with tuberculin is of value. The course usually commences with very small doses and proceeds by rapid increases till large doses are tolerated, care being taken not to produce a febrile reaction ; the doses are given at intervals of three to four days. The following represents a scheme of dosage commonly employed.

Injections are given at intervals of three to four days beginning with 0·00001 c.c., increasing to 0·00002 c.c., then to 0·00004 c.c. and so on until eventually a dose of 1 c.c. of undiluted tuberculin may be reached ; the dose is not increased if any reaction occurs. If a relapse occurs after such a course of treatment the previous course may be repeated.

DILUTIONS OF TUBERCULIN (T.)

It was formerly stated that tuberculin rapidly loses its potency on dilution. It is, however, impossible by titration in the laboratory to detect deterioration in dilutions of Old Tuberculin kept at ordinary room temperature for several weeks. Dilutions of Old Tuberculin (T.) to the simple negative powers of 10 only are prepared at The Wellcome Physiological Research Laboratories to special order. They are supplied in Great Britain, Northern Ireland and Eire only. The dilutions should not be used after the date indicated on the package.

UNDILUTED TUBERCULINS

Undiluted Tuberculins retain their potency for long periods. The time-limit indicated on the package is given to comply with the Government regulations of certain countries.

TUBERCULIN P.P.D. PURIFIED PROTEIN DERIVATIVE

P.P.D. or Purified Protein Derivative may be regarded as the active principle of tuberculin and is prepared by a method resembling that described by Seibert, involving ultrafiltration and precipitation with trichloracetic acid.

P.P.D. is prepared for issue in the following manner. The dry powder is dissolved in a special buffer solution. The potency of the material is checked on tuberculous guinea-pigs to ensure that it is equivalent to International Standard Old Tuberculin, *i.e.* the official English standard. The standardised solution is then diluted in a special borate buffer solution, the final dilutions being distributed in bottles which contain 1 c.c. (ten doses of 0·1 c.c.) of strengths equivalent to 1 : 10,000 (0·1 c.c. of this strength corresponding to 0·00005 mgm. of P.P.D.), 1 : 1000, and 1 : 100 of Standard Tuberculin. These dilutions are evaporated to dryness *in vacuo* under sterile conditions.

Directions for Use.—Immediately before use 1 c.c. of the special borate solvent, which is supplied with the " dried dilutions," is added to the required bottle by means of the sterile syringe and needle that are to be used in the test. This bottle is left stoppered for two minutes for the " dried dilution " to dissolve and is then well shaken.

The dose of this solution is 0·1 c.c., injected intradermally, preferably in the middle of the left forearm.

P.P.D. possesses the following advantages :—(1) Constancy of composition and potency because an exact quantity by weight of the effective substance is always injected : (2) absence of non-specific substances : (3) stability in the dry state ; and (4) ease of preparation of dilutions.

Tuberculin P.P.D. in Diagnosis.—The usual initial dose recommended when there is a possibility of active tuberculosis is 0·1 c.c. of dilution 1 : 10,000. It has been stated that if this amount gives a negative reaction it is unnecessary to inject 0·1 c.c. of 1 : 1000 and one may safely proceed to 0·1 c.c. of 1 : 100 ; the patient is regarded as negative only if this dose causes no reaction. Other investigators have used 0·1 c.c. of 1:1000 as the initial routine dose and it is agreed that the stronger the dilution of tuberculin used the more positives will be obtained.

Stability.—In the dried state dilutions are very stable and have shown no detectable loss after several years at ordinary room temperature. *When redissolved, however, they deteriorate more rapidly and it is a wise precaution to use them on the day of preparation.*

To facilitate subsequent reference to Tuberculin products, it is particularly desired that the physician should note on the chart

or in his case-book, the series number given on the wrapper and label. In the case of tuberculin dilutions the date of the dilution should be noted.

Important Note.—Attention is directed to the following:—"Adherence of Tuberculin to Syringes, etc.—Tuberculin is exceptionally heat-stable and adheres very firmly to filter candles, glass-ware and all other laboratory and ward equipment with which it comes in contact. Every possible precaution must therefore be taken to prevent apparatus which has once been used for tuberculin from ever being employed for other biological work. Cleaning with strong chromic acid solution appears to be effective for removing traces of tuberculin from certain apparatus if it is allowed to act for 48 hours or longer. It is preferable, however, *to reserve syringes not only for tuberculin but for each dilution to be injected in the Mantoux Tests*. Fry has published a valuable note on the fallacies introduced by the indiscriminate use of syringes for Schick and tuberculin testing. Traces of tuberculin adherent to syringes may give rise to false Schick positive reactions, and consequently to the impression that a diphtheria prophylactic has failed to immunise satisfactorily."

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PRICE LIST

OF

'WELLCOME'

BRAND

SERA, VACCINES AND TUBERCULINS

PREPARED AT

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES

BECKENHAM, KENT

SUPPLIED BY

BURROUGHS WELLCOME & CO.

(The Wellcome Foundation Ltd.)

LONDON

Associated Houses :

New York Montreal Sydney Cape Town Bombay Shanghai Buenos Aires

ORDERING

Orders may be sent by telegram. A marginal code word appears against the product it indicates and code words for sizes and quantities are as follows:

	One	Three	Six	Twelve
Regular size ..	ARYCE	ASATA	ASEDO	ASEZA
10 c.c. ..	ENEWE	ENEYU	ENEZA	ENGRA
25 c.c. ..	ENOBA	ENOGU	ENRIO	ENTAO

EXAMPLES :—

Send six containers of 'Wellcome' Refined Diphtheria Antitoxin —Globulins, each of 5000 units = ASEDO NUNOK

Send five containers of 'Wellcome' Anti-meningococcus Serum, each of 25 c.c. = FIVE HISUK ENOBA

Please use PLAIN CAPITAL LETTERS *to avoid errors in transmission*

To facilitate subsequent reference to these products, it is particularly desired that the physician should note on the chart, or in his case-book, the series number given on the wrapper and label.

STORAGE OF SEROLOGICAL PRODUCTS

The optimum temperature for storage is just above freezing point, *i.e.* 2°–4° C., the loss of potency of sera being then probably much less than 5 per cent per annum. At higher temperatures the rate of deterioration increases. Thus in a cool cupboard (not above 15° C.), in a room in temperate zones, it may be between 5 and 10 per cent, while in a hot cupboard or in the tropics it may easily exceed 25 per cent per annum.

WARNING

It is most important that serological products containing antiseptic should not be frozen solid. During freezing and also during thawing, there is a temporary high concentration both of protein and of antiseptic. The increase in concentration of the antiseptic may cause considerable deterioration of the serological product, although with undiluted serum this loss of potency may be very small.

PURCHASE TAX

All 'Wellcome' Sera, Tuberculins and Vaccines are exempt from Purchase Tax.

'WELLCOME' BRAND
THERAPEUTIC AND PROPHYLACTIC SERA

REFINED DIPHTHERIA ANTITOXIN—GLOBULINS

Potency not less than 4000 Units per c.c. 500 to 4000 unit strengths are adjusted to 1 c.c. for convenience of administration.

	Each	Telegraphic Code
Ampoules of 500 Units	1/6	NUMUH
„ „ 1000 „	2/0	NUMAX
„ „ 2000 „	3/3	NUNEC
„ „ 4000 „	6/0	NUNIS
„ „ 5000 „	7/6	NUNOK
„ „ 6000 „	8/9	NUNUT
„ „ 8000 „	9/6	NUPAB
„ „ 10,000 „	11/6	NUPET
„ „ 20,000 „	21/6	NUPOM
„ „ 40,000 „	40/0	NUPUZ
„ „ 60,000 „	58/6	PEZON

ANTI-DYSENTERY (SHIGA)

Potency 10,000 Units in 10 c.c. or less

Ampoules of 10,000 Units	8/6	HITAX

REFINED GAS-GANGRENE ANTITOXIN (PERFRINGENS)—GLOBULINS

(Cl. perfringens = Cl. welchii)

Potency 1600 Units in 1 c.c. or less

Rubber-capped bottles of 4000 Units	6/6	NUZIB
Rubber-stoppered „ „ 10,000 „	15/0	NUZOX

REFINED GAS-GANGRENE ANTITOXIN—GLOBULINS (POLYVALENT)

For Prophylactic Use

Cl. perfringens Antitoxin (=*Cl. welchii*)	3000 Units	Ampoules of 4 c.c. or less	8/0 PAMEX
Cl. septique „	1500 „		
Cl. œdematiens „	1000 „		

REFINED GAS-GANGRENE ANTITOXIN—GLOBULINS (POLYVALENT)

For Therapeutic Use

Cl. perfringens Antitoxin (=*Cl. welchii*)	7500 Units	Ampoules of 10 c.c. or less	18/0 PAMAT
Cl. septique „	3750 „		
Cl. œdematiens „	2500 „		

HORSE, NORMAL, No. 1 — HITUL

Contains 0·4 per cent Tricresol

Ampoules of 10 c.c.	1/6
„ 25 c.c.	3/0

	Each	Telegraphic Code
***HORSE, NORMAL, No. 2**		
In bulk for media-making		
Rubber-stoppered bottles of 250 c.c. ..	7/6	LOHIF
HORSE, NORMAL, No. 3		
Fresh, unheated (without preservative), less than 48 hours old. No sterility or toxicity tests. Guarantee as for No. 2. Orders for Normal Horse Serum No. 3 must reach us two clear working days before it is required. Not less than 100 c.c. supplied.		
Rubber-stoppered bottles of 100 c.c. ..	9/0	JUMES
ANTI-LEPTOSPIRA		
Ampoules of 10 c.c.	3/6	NOLUV
ANTI-MENINGOCOCCUS		HISUK
Ampoules of 10 c.c.	3/6	
„ „ 25 c.c.	8/6	
CONCENTRATED ANTI-PNEUMOCOCCUS, TYPE I		
Ampoules of 20,000 International Units in 12 c.c. or less	30/0	NIKIG
CONCENTRATED ANTI-PNEUMOCOCCUS, TYPE II		
Ampoules of 20,000 International Units in 12 c.c. or less	30/0	NISON
CONCENTRATED ANTI-PNEUMOCOCCUS, TYPES I + II		
Ampoules of 10,000 International Units of each type in 12 c.c. or less	30/0	NOGUZ
REFINED STAPHYLOCOCCUS ANTITOXIN—GLOBULINS		
1000 Units in 1 c.c. or less		
Ampoules of 2500 Units	6/0	JOLEB
„ „ 20,000 „	41/0	JOYUS
REFINED STREPTOCOCCUS ANTITOXIN (SCARLATINA)—GLOBULINS		
1000 U.S.A. Units in 1 c.c. or less		
Ampoules of 1500 U.S.A. Units ..	7/0	PEDUD
„ „ 3000 „ „ ..	12/6	PAFEM
„ „ 10,000 „ „ ..	32/0	PAGEK
ANTI-STREPTOCOCCUS, ERYSIPELAS		
Ampoules of 25 c.c.	8/6	HIPOB
ANTI-STREPTOCOCCUS, POLYVALENT		HIPIM
Ampoules of 10 c.c.	3/6	
„ „ 25 c.c.	8/6	

*This contains no antiseptic, but has been heated to 58° C. for two hours. It is not tested for sterility or toxicity, and is specially intended for the preparation of bacteriological media sterilised by heat. The only guarantee given is that the serum is obtained from a healthy horse, with the usual aseptic precautions.

	Each	Telegraphic Code
ANTI-STREPTOCOCCUS, PUERPERAL FEVER		HIRAZ
Ampoules of 10 c.c.	**3/6**	
„ „ 25 c.c.	**8/6**	
REFINED TETANUS ANTITOXIN—GLOBULINS		
For Prophylactic Use		
Ampoules of 1000 International Units (=500 U.S.A. Units) in 1 c.c. ..	**1/2**	PANIR
Ampoules of 3000 International Units (=1500 U.S.A. Units) in 1 c.c. ..	**2/6**	PANOH
Rubber-capped bottles of 10 doses of 1000 International Units (=500 U.S.A. Units) in 10 c.c.	**7/2**	PANUB
Rubber-capped bottles of 10 doses of 3000 International Units (=1500 U.S.A. Units) in 10 c.c. (1 c.c. per dose) ..	**20/6**	PAPOZ
REFINED TETANUS ANTITOXIN—GLOBULINS		
For Therapeutic Use		
Rubber-capped bottles of 20,000 International Units (10,000 U.S.A. Units).. 8000 International Units in 1 c.c. or less	**13/9**	PAGIB
REFINED TETANUS AND GAS-GANGRENE POLYVALENT ANTITOXIN—GLOBULINS		
For Prophylactic Use		
Cl. tetani Antitoxin 3000 International Units *Cl. perfringens* „ (=*Cl. welchii*) 3000 Units *Cl. septique* „ 1500 „ *Cl. œdematiens* „ 1000 „ Ampoules of 5 c.c. or less	**10/0**	PAPUH

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	Each	Telegraphic Code
DIPHTHERIA PROPHYLACTIC A.P.T. ALUM PRECIPITATED TOXOID		
Ampoules of 0·5 c.c.	**1/0**	NOZEM
Boxes of 100 ampoules of 0·5 c.c... ..	**95/0**	
Rubber-capped bottles of 1 c.c.	**1/6**	NOMOM
Boxes of 100 ampoules of 1 c.c.	**145/0**	
Rubber-capped bottles of 5 c.c.	**4/3**	
DIPHTHERIA PROPHYLACTIC T.A.F. TOXOID-ANTITOXIN FLOCCULES (SUSPENSION)		NEKIF
Rubber-stoppered bottles of 1 c.c... ..	**1/6**	
Set of three	**4/6**	PANEY
Rubber-capped bottles of 10 c.c. ..	**8/6**	
„ „ „ „ 25 c.c. ..	**19/6**	

	Each	Telegraphic Code
DIPHTHERIA PROPHYLACTIC T.A.M. TOXOID-ANTITOXIN MIXTURE		MAVIT
Rubber-stoppered bottles of 1 c.c. ..	1/6	
Rubber-capped „ „ 5 c.c. ..	4/3	
„ „ „ „ 10 c.c. ..	7/6	
„ „ „ „ 25 c.c. ..	17/6	
SCHICK TEST PRODUCTS		
DILUTED TOXIN (TEST) AND HEATED TOXIN (CONTROL)		
	per set	
1 c.c. sets (rubber-capped bottles) ..	2/6	MAVED
5 c.c. „ („ „ „) ..	8/6	MIGAC
10 c.c. „ („ „ „) ..	12/6	MIGIX
SCARLET FEVER PROPHYLACTIC (A)		MOZIW
Containing 2500 skin test doses per c.c.		
	Each	
Rubber-capped bottles of 1 c.c. ..	1/6	
„ „ „ „ 10 c.c. ..	7/6	
„ „ „ „ 25 c.c. ..	17/6	
SCARLET FEVER PROPHYLACTIC (D)		NOSAM
Containing 50,000 skin test doses per c.c.		
Rubber-capped bottles of 1 c.c. ..	2/0	
„ „ „ „ 10 c.c. ..	12/0	
„ „ „ „ 25 c.c. ..	28/0	
DICK TEST PRODUCTS		
DILUTED SCARLET FEVER TOXIN (TEST) and HEATED SCARLET FEVER TOXIN (CONTROL)		
	per set	
1 c.c. sets (rubber-capped bottles) ..	1/6	MOVEN
5 c.c. „ („ „ „) ..	6/6	MOVID
SCHULTZ-CHARLTON TEST SOLUTION		MUNAY
	Each	
Rubber-capped bottles of 1 c.c.	2/0	
„ „ „ „ 5 c.c.	7/0	
STAPHYLOCOCCUS TOXOID A (1/10 DILUTION)		NONAG
Rubber-capped bottles of 1 c.c.	1/6	
„ „ „ „ 5 c.c.	4/3	
STAPHYLOCOCCUS TOXOID B (UNDILUTED)		NONER
Rubber-capped bottles of 1 c.c.	1/6	
„ „ „ „ 5 c.c.	4/3	
TETANUS TOXOID		NURER
Rubber-stoppered bottles of 1 c.c. ..	1/6	
Rubber-capped „ „ 5 c.c. ..	4/3	

'WELLCOME' BRAND PRODUCTS FOR LABORATORY DIAGNOSIS

	Each	Telegraphic Code
SACHS-GEORGI ANTIGEN		
For use in the Sachs-Georgi Test for the diagnosis of syphilis.		
Containers of 1 c.c.	1/3	MAWOR
WASSERMANN ANTIGEN		
Alcoholic Extract of Sheep's Heart with Cholesterol.		
Containers of 1 c.c.	1/3	LAHER
Containers of 10 *c.c. supplied to special order*		
HÆMOLYTIC SERUM (GLYCERINATED) FOR SHEEP'S CORPUSCLES		
For use in the Wassermann and other complement-deviation tests.		
Rubber-stoppered bottles of 1 c.c... ..	2/6	LAHAP
Containers of 10 *c.c. supplied to special order*		
SERA FOR AGGLUTINATION TESTS AGAINST THE FOLLOWING ORGANISMS		
V. choleræ		LUNEX
Bact. dysenteriæ (Shiga)		LUNIH
Bact. dysenteriæ (Flexner)		LUNOD
Bact. enteritidis (Gaertner)		MABAF
Br. melitensis		LUNUS
Bact. paratyphosum A		LUZOP
Bact. paratyphosum B		LUZUL
Str. pneumoniæ Type I		MOZEH
Str. pneumoniæ Type II		NIBUF
Bact. typhosum		LUPIT
Each in rubber-stoppered bottles of 1 c.c...	2/0	
Also supplied in containers of 10 *c.c. to special order*		
SUSPENSIONS FOR AGGLUTINATION TESTS OF THE FOLLOWING ORGANISMS		
V. choleræ		LUPOV
Bact. dysenteriæ (Shiga)		LUPUF
Bact. dysenteriæ (Flexner)		LURIN
Bact. enteritidis (Gaertner)		MABOC
Br. melitensis		LURUM
Bact. paratyphosum A		MABEB
Bact. paratyphosum B		MABIM
Bact. typhosum		LUSAP
Each in rubber-stoppered bottles of 1 c.c...	2/6	
Also supplied in containers of 10 *c.c. to special order*		

	Each	Telegraphic Code
BLOOD-GROUPING SERA		
Sets containing one capillary tube each Group A (Moss II) White and Group B (Moss III) Yellow	net 1/0	NOSOW
BLOOD-GROUPING SERUM GROUP A (MOSS II)		
Rubber-stoppered bottles of 1 c.c... ..	10/0	PIGAY
BLOOD-GROUPING SERUM GROUP B (MOSS III)		
Rubber-stoppered bottles of 1 c.c... ..	10/0	PIGEL
PRECIPITATING SERUM, ANTI-HUMAN		
Ampoules of 1 c.c.	7/6	NIBAP
Other Precipitating Sera available on special request		

'WELLCOME' BRAND VACCINES

The following products, except where otherwise stated, are supplied in three packings:—

		Telegraphic Code
Ampoules of 1 c.c.	1/9 each	
Rubber-capped bottles of 10 c.c.	10/3 ,,	
,, ,, ,, of 25 c.c.	24/0 ,,	
ACNE, MIXED, No. 1		KIMIN
Each c.c. contains 10 million *Acne Bacillus* 250 ,, *Staphylococcus*, mixed		
ACNE, MIXED, No. 2		LOFIW
Each c.c. contains 125 million *Acne Bacillus* 125 ,, *Staphylococcus*, mixed		
ACNE, MIXED, No. 3		LOFUX
Each c.c. contains 500 million *Acne Bacillus* 500 ,, *Staphylococcus*, mixed		
V. CHOLERÆ		LUFEY
4 mgm. (approx. 10,000 million organisms) per c.c.		
BACTERIUM COLI		
10 million organisms per c.c.		KEFAN
50 ,, ,, ,, ,,		KEFIP
250 ,, ,, ,, ,,		LOGUW
1000 ,, ,, ,, ,,		LOHAD

See note on previous page

		Telegraphic Code
CORYZA		
Each c.c. contains		LUNAW
50 million	*Corynebacterium hofmanni*	
50 „	*Bacterium friedländeri*	
50 „	*Neisseria catarrhalis*	
50 „	*Hæmophilus influenzæ*	
50 „	*Staphylococcus*, mixed	
10 „	*Pneumococcus*	
10 „	*Streptococcus*, mixed	
GONOCOCCUS		
5 million	organisms per c.c.	LAKEG
20 „	„ „ „	KEHUF
200 „	„ „ „	KEGOP
1000 „	„ „ „	JUNOS
INFLUENZA, MIXED		MAPIS
Each c.c. contains		
400 million	*Hæmophilus influenzæ*	
200 „	*Pneumococcus*	
80 „	*Streptococcus*	
BR. MELITENSIS (MEDITERRANEAN FEVER)		KOKEW
100 million	organisms per c.c.	
PNEUMOCOCCUS		
10 million	organisms per c.c.	KEDEW
50 „	„ „ „	KEDIF
M. RHEUMATICUS		
10 million	organisms per c.c.	LABAM
50 „	„ „ „	LABIH
STAPHYLOCOCCUS AUREUS		
200 million	organisms per c.c.	JUGAS
1000 „	„ „ „	JUGER
STAPHYLOCOCCUS, MIXED		
200 million	organisms per c.c.	KEFOY
1000 „	„ „ „	JOYAF
STREPTOCOCCUS, POLYVALENT		
10 million	organisms per c.c.	KEDOK
50 „	„ „ „	KEDUP
ANTI-TYPHOID-PARATYPHOID (T.A.B.)		MACIC
Each c.c. contains		
1000 million	*Bact. typhosum*	
750 „	*Bact. paratyphosum A*	
750 „	*Bact. paratyphosum B*	
Also supplied in rubber-capped bottles of 1·5 c.c. (sufficient for one immunising course) .. .. .. each	**2/3**	TSOC

	Each	Telegraphic Code
ANTI-TYPHOID-PARATYPHOID A, B and C (T.P3)		ITTOR
Each c.c. contains		
1000 million *Bact. typhosum*		
750 „ *Bact. paratyphosum A*		
750 „ *Bact. paratyphosum B*		
750 „ *Bact. paratyphosum C*		
Supplied only in special containers of 1·5 c.c. (sufficient for one immunising course) . .	**2/3**	
and rubber-capped bottles of 25 c.c. . .	**24/0**	
ANTI-TYPHOID-PARATYPHOID A and B and CHOLERA		
Each c.c. contains		
1000 million *Bact. typhosum*		
750 „ *Bact. paratyphosum A*		
750 „ *Bact. paratyphosum B*		
10,000 „ (= 4 mgm.) *V. choleræ*		
Supplied only in rubber-capped bottles of 1 c.c.	**1/9**	MADUD
ANTI-TYPHOID-PARATYPHOID VACCINE with TETANUS TOXOID (T.A.B.T.)		
First Dose (No. 1)—1 c.c. Tetanus Toxoid containing		
500 million *Bact. typhosum*		
375 „ *Bact. paratyphosum A*		
375 „ *Bact. paratyphosum B*		
Second Dose (No. 2)—1 c.c. Tetanus Toxoid containing		
1000 million *Bact. typhosum*		
750 „ *Bact. paratyphosum A*		
750 „ *Bact. paratyphosum B*		
Sets of 1 c.c. ampoules of No. 1 and No. 2 sufficient for one immunising course . . . . . per set **4/0**		ITSUL
Rubber-capped bottles of 25 c.c. each of No. 1 and No. 2 . . per set **56/0**		PEZUP
WHOOPING COUGH (*H. pertussis*)		
10,000 million organisms per c.c.		
Rubber-capped bottles of 10 c.c.	**10/3**	NUDAS
Sets of four 0·5 c.c. ampoules, comprising one complete immunising course, per set	**5/0**	PETOS
OPACITY TUBES		
For the Standardisation of Bacterial Vaccines		
Sets of 10 tubes (in cardboard outer) . .	**33/0**	MORIS

	Each	Telegraphic Code
'WELLCOME' BRAND TUBERCULINS		
UNDILUTED TUBERCULINS—EXOTOXIC		
Old Tuberculin, Human (T.)		LAPEW
Rubber-stoppered bottles of 1 c.c. ..	**1/6**	
„ „ „ „ 5 c.c. ..	**6/0**	
Old Tuberculin, Bovine (P.T.)		LARAV
Rubber-stoppered bottles of 1 c.c. ..	**1/6**	
„ „ „ „ 5 c.c. ..	**6/6**	
Tuberculin Bouillon Filtrate, Human (T.O.A.)		LASAG
Rubber-stoppered bottles of 1 c.c. ..	**1/6**	
„ „ „ „ 5 c.c. ..	**6/0**	
Tuberculin Bouillon Filtrate, Bovine (P.T.O.)		LATAT
Rubber-stoppered bottles of 1 c.c. ..	**1/6**	
„ „ „ „ 5 c.c. ..	**6/6**	
TUBERCULIN DILUTIONS		
Fresh sterile dilutions of Old Tuberculin T., to the simple negative powers of 10 *(e.g.*, 0·01, 0·001, 0·0001 c.c.) are supplied to order in containers of 1 c.c.		
Old Tuberculin (T.)		
Dilutions up to and including 0·1 c.c. in 1 c.c. ampoules	**2/0**	
TUBERCULIN FOR DIAGNOSIS ONLY		
Tuberculin (Human)—For von Pirquet Cutaneous Reaction		
Three capillary tubes of Old Tuberculin Human (T.) and three of Control in each carton .. .. Per carton	**4/0**	KOLAF
PURIFIED PROTEIN DERIVATIVE		
Rubber-stoppered bottles of dried P.P.D. and solvent to make 1 c.c. (10 test doses) solutions equivalent to dilutions of Standard Tuberculin of the potency indicated in parentheses		
P.P.D. No. 1. (1 in 10,000)	**4/3**	NUSIF
„ No. 2. (1 in 1000)	**4/3**	NUSOP
„ No. 3. (1 in 100)	**4/3**	NUSUW

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